A PHYSIOLOGICAL STUDY OF THE GROWTH OF THE MEDITERRANEAN FLOUR MOTH (EPHESTIA KUEHNIELLA ZELLER) IN WHEAT FLOUR

DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science of Columbia University

By

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A PHYSIOLOGICAL STUDY OF THE GROWTH OF THE MEDITERRANEAN FLOUR MOTH (EPHESTIA KUEHNIELLA ZELLER) IN WHEAT FLOUR[1]

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INTRODUCTION

Although much valuable information has been gathered in regard to the natural food substances necessary for the growth of many species of insects, there is still a need for additional information on the quantity and quality of proteins, carbohydrates, fats, salts, vitamins, and other substances necessary for their growth and maintenance. Such studies would be of interest from the standpoint of comparative physiology, and would give knowledge of value in the control of insect pests. Poisoned food preparations are now used as baits to control such insects as cockroaches, crickets, grasshoppers, cutworms, house flies, and ants, and it is quite possible that investigations of the food requirements of these and perhaps other insects would lead to the adoption of baits of greater efficiency than those now employed. The present results are offered as a contribution to some of these problems.

The modern knowledge of vitamins has been largely derived from feeding experiments on mammals and birds, especially on the rat, the guinea pig, and the pigeon, but within recent years the investigations have been extended to include certain protozoa, insecta, batrachia, and additional species of birds and mammals. Vitamins or vitaminlike substances have been found also in certain bacteria, yeasts, and higher plants (*12*)[3]. The chemical nature of vitamins is still in doubt. They are recognized by their solubility in various reagents, their curative effects on animals suffering from certain deficiency diseases, and their stimulating powers on growth. They occur in nature associated with other substances, and it is improbable

[1] Received for publication July 14, 1925; issued May, 1926. The investigation reported here was begun in the fall of 1917 and continued with some interruptions until May, 1921. A brief summary of the results was presented before the Entomological Society of America at Toronto, Canada, December 28, 1921; and the results have also been referred to by Harrow (*16, p. 115*). Publication of the present paper has been delayed through unavoidable circumstances in connection with postwar conditions.

[2] The writer wishes to acknowledge his indebtedness to Prof. William J. Gies, of Columbia University, for his guidance and suggestions; to G. F. White, of the Bureau of Entomology, who has rendered much assistance in the control of a protozoan disease of the moth larvae; and to P. W. Whiting, of the University of Maine, T. J. Headlee, State entomologist of New Jersey, and Z. P. Metcalf, of the North Carolina State College of Agriculture and Engineering, who have furnished the writer entomological material.

[3] Reference is made by number (italic) to "Literature cited," p. 927.

that they have yet been isolated in a pure condition, although several highly concentrated vitamin preparations have been reported.

Not all animals respond equally to a given concentration of a vitamin-containing preparation, the amounts necessary for growth, maintenance, and reproduction varying considerably, in some cases, from species to species. For instance, rats and mice and many species of birds seem to grow normally in the absence, or at least with only a very low concentration, of vitamin C, whereas guinea pigs rapidly succumb unless this vitamin is present in the food.

Of the vitamins now recognized as essential for the growth of higher animals, probably only two exist in an active condition in the wheat kernel, namely, vitamin A and vitamin B. Dry cereals apparently do not possess vitamin C in an active state.

VITAMIN STUDIES ON INSECTS

The studies on insects which have a bearing on the influence of vitamins in nutrition have largely been made on the fruit fly *Drosophila melanogaster* (*ampelophila*) Loew. Loeb and Northrop (*20*) raised 12 successive generations of Drosophila under aseptic conditions upon a medium of sterile yeast, citric acid, and water. Casein, edestin, egg albumin, milk, or a mixture of amino acids, when supplemented with a salt mixture and sucrose, did not permit growth to the pupal stage. The substance in yeast which promoted growth was fairly heat-stable. When the yeast was extracted with hot or cold alcohol it could not be utilized as food, nor would a mixture of the extracted yeast and the alcoholic extract support the growth of the larvae. The addition of butter, nucleic acid, thymus, or thyroid extract to the culture medium likewise did not permit growth. Those writers concluded that "the necessary substance must be different from that needed for pigeons, rats, and other warm-blooded animals." Northrop (*25*) believes that the yeast plant supplies Drosophila larvae with growth-promoting substances similar to vitamins, which the plant is able to synthesize from simple chemical compounds of known structure. He obtained normal growth and development with such glandular tissues as liver, pancreas, and kidney, and also with dead adult Drosophila, but spleen, heart muscle, skeletal muscle, blood, adrenal, thyroid, and thymus glands, testes, tumor tissue from a mouse, and tethelin resulted at most in abnormal growth. In a later study Loeb and Northrop (*21*) found that while the larvae required yeast, the adult flies could live for a relatively long time on a medium consisting of beef broth, agar, peptone, egg, and glucose. It was thought that the vital substances found in yeast were not required for maintenance, or were needed in such small amounts that they could be supplied by the hydrolytic processes which take place in the cells of the adult. Northrop (*26*) observed that a deficiency in yeast caused a retardation of growth during the larval period, but the length of life of the adult fly was not affected.

Guyénot (*15*), in an article which is the culmination of an extensive series of experiments on the growth of *Drosophila melanogaster*, concludes that sterilized yeast can be replaced by an artificial medium consisting of salts, peptone, lecithin, and an undetermined substance present in the alcoholic extracts, bouillons, and autolysates of yeast and liver tissue. The substance in yeast which is indispensable for

the life of Drosophila is soluble in water, insoluble in cold 100 per cent alcohol, soluble in boiling 100 per cent alcohol, from which it is precipitated on cooling, and soluble in 90, 80, and 70 per cent alcohols. It resembles in certain respects the water-soluble vitamin B necessary for the normal life of higher animals.

Baumberger (*3*) succeeded in rearing adult *Drosophila melanogaster* upon a medium consisting of yeast nucleoprotein, carbohydrates, and salts. He believes that "the larvae are dependent on the nucleoprotein of yeast for special substances necessary in their growth" and that lecithin is unnecessary for normal development. The nucleoprotein was washed first with acid alcohol, then with neutral alcohol, and finally extraced with ether to free it of impurities.

Bacot and Harden (*2*) grew larvae of *Drosophila melanogaster* aseptically on a basal diet of purified casein, starch, sucrose, agar, and a salt mixture, to which the various vitamin preparations were added. In the presence of butterfat (vitamin A) and yeast extract (vitamin B), growth occurred irrespective of the presence or absence of lemon juice (vitamin C). When butterfat was added, growth occurred if yeast extract was present, but failed in its absence. Here also growth seemed not to depend upon the presence or absence of lemon juice. In media containing yeast extract with or without lemon juice, growth occurred when as little as two drops of butterfat was added, but did not take place in its absence. When casein was omitted from the diet, the nitrogen furnished by the yeast extract was insufficient for the growth of the larvae. Vitamin B from wheat germ also permitted growth in the presence of two drops of butterfat. Those writers concluded that complete development of Drosophila requires the presence of vitamin B, and very small quantities, if any, of vitamin A. The antiscorbutic vitamin (C) is not essential for growth and development.

Glaser (*13*) studied the effect of food on the longevity and reproduction of *Musca domestica* L., *Stomoxys calcitrans* L., and *Lyperosia irritans* L. He mentions briefly the intestinal bacteria of adult flies as a possible source of vitamins during the adult stage. Elsewhere the same writer describes experiments (*14*) in which larvae of *Drosophila melanogaster* grew and became adults that laid many fertile eggs on sterile media to which small quantities of fresh sterile orange and grapefruit juices were added daily. It was thus shown that Drosophila can live on fruit substances without the aid of microorganisms, and in the absence of products liberated from the dead bodies of microorganisms.

Wollman (*35*) grew larvae of *Calliphora vomitoria* aseptically upon brain tissue which had been sterilized in an autoclave at 130° C. for 45 minutes. He advanced these three hypotheses to account for this fact: Either the vitamin of the brain tissue was not destroyed at the temperature used, or the larvae did not require vitamins for growth, or they may have synthesized vitamins from materials in the medium. In another article (*36*) he proved that a temperature of 134° to 135° C. for 1½ hours (using an autoclave) did not destroy all the vitamin in brain tissue necessary for the growth of rats, and that Calliphora larvae grown aseptically on brain tissue sterilized at a high temperature furnished an appreciable amount of vitamin when fed to young rats. He concludes that the larvae do not produce vitamin, but accumulate and concentrate the vitamin which they obtain from the sterilized brain tissue. More recently Wollman (*37*) has been able

to grow larvae of the house fly (*Musca domestica* L.) aseptically upon horse manure sterilized at a low temperature.

Portier (*31*) reared larvae of *Tenebrio molitor* on a mixture of bran and flour sterilized at 130° C. in an autoclave for 45 minutes. He interprets his results as follows: Symbiotic microorganisms, present in foods, are responsible for the synthesis of the special substances necessary for life. Certain species of insects, of which Tenebrio is one, have normally a supply of symbiotic microorganisms in the epithelial cells of the intestine. The symbionts are passed from the parent insect to its offspring by means of the egg. Other species of animals derive their necessary symbiotic microorganisms from the food. When the food is sterilized at a temperature sufficiently high to kill the symbionts, the species like Tenebrio which possess symbiotic organisms within the intestinal epithelium grow normally, while those species not so endowed cease to grow and ultimately die.

Aeppler (*1*) investigated the vitamin content of the food of the larval honeybee, *Apis mellifera* L. It did not contain sufficient vitamin A to support the growth of young rats at the normal rate. Vitamin B was present, however, in amounts sufficient for the normal growth of rats. Since it has been shown by Hawk, Smith, and Bergeim (*17*) that honey contains very little vitamin B, it is believed that the amount present in the larval food is derived from the pollen[4].

From the foregoing publications it seems to be an established fact that vitamin B is necessary for the normal growth of certain insects, particularly *Drosophila melanogaster*. Baumberger's results (*3*) are rather opposed to this view, but there is a possibility that the yeast used by him contained sufficient vitamin B, even after purification, for the growth of the larvae. Yeast is one of the richest known sources of this vitamin, and, according to Osborne, Wakeman, and Ferry (*30*), certain proteins are freed from it with difficulty.

The rôle of vitamin A is not so clearly defined, but from the work of Bacot and Harden (*2*) there is reason for believing it a necessary constituent of the food of insects. The present work lends strong support to this view. The investigations on Drosophila also show that not more than traces of fats in any form are necessary in the diet of this insect.

The information at present available leads to the conclusion that vitamin C is not essential for the normal growth and development of insects.

LIFE HISTORY OF EPHESTIA KUEHNIELLA

The Mediterranean flour moth belongs to the lepidoterous family Pyralidae, and is the commonest of the species of Ephestia found in North America. It occurs wherever flour and other cereal foods are

[4] The report of an interesting study of the nutrition of the flour beetle (*Tribolium confusum*) by Chapman (*7*) has recently appeared. It is found that the lower grades of wheat products (low-grade flour and bran) slightly retard growth and metamorphosis, whereas rice flour made from polished rice slightly retards growth but has a marked effect upon metamorphosis. When all the embryo is removed, the effect of the rice flour is still more pronounced. Oat, rye, and corn flours also retard growth slightly and metamorphosis more, while barley flour resembles the best grades of wheat flour in its effect. The effects of a number of synthetic mixtures on growth and transformation are also given. Chapman concludes that wheat embryo is more satisfactory for growth and transformation than the other substances tried, that vitamin B from wheat embryo does not seem to supplement deficient diets, and that the requirements for growth, in the sense of increasing size, are less exacting than for maturity or transformation. Vitamin A does not seem to have been present in any of these food mixtures, except in one which contained wheat embryo.

stored or manufactured, and often causes serious damage in mills and warehouses.

Fertilized females generally lay their eggs from 24 to 48 hours after emergence. Females laying infertile eggs and virgin females require a longer time for oviposition than is usual for fertile moths. The eggs are normally laid during the night and are placed in the food material, but when food is absent they may be scattered indiscriminately about the walls of the container in which the moths are placed. According to Burkhardt (*5*) the female lays between 150 and 200 eggs. Small females lay fewer. Under the conditions of these experiments, the incubation period varied from 4 to 11 days, depending on the temperature. During the cooler part of the year the average period was 6 days.

The newly-hatched larva measures about 1 mm. in length, and weighs about 0.023 mgm. (mean weight of 150 larvae). It begins feeding at once, and spins a silken tube as it works its way through the food substance. The amount of silk produced gives a rough idea of the extent to which growth has taken place. In normal food the larvae attain a length of about 12 mm. and a weight of about 24.5 mgm. before pupation. The growth rate varies with the temperature, kind of food, and possibly other factors.

Various observers have stated the duration of the pupal stage as from 8 to 16 days at ordinary summer temperature, but under adverse conditions of temperature it may be greatly extended.

The moth lives but a short while after emergence, and apparently it takes no food during that time. The mean length of life under laboratory conditions for 30 males was 9.5 days and for 34 females was 8.5 days, while the maximum duration of life was 14 and 15 days, respectively.

Under conditions of apparently normal nutrition and environment, great variations in the fertility of the moths have been observed. Whiting (*34*) found that usually not more than 5 or 10 per cent of the pairs of moths were fertile under experimental conditions, though occasionally the percentages rose to as high as 50, 60, or even 70. In one instance the writer observed a fertility of 90.5 per cent in 42 pairs under experimental conditions, but often the ratio is lower than this. In view of this fact, the question of the effect of food upon fertility has not been considered at this time.

The sex ratio of moths, as based on 1,382 individuals, was found to be 47.5 per cent males and 52.5 per cent females.

Although the moths are easily reared in a small space, considerable care is necessary to prevent the spread of disease among them. The larvae are subject to infection by a protozoan parasite which often causes a heavy mortality and may at times kill all the larvae in a bottle. However, the larvae which complete growth and transformation in the infested bottles emerge as moths in approximately normal time. Sterilization of the bottles in which the larvae are grown, and selection of moths from disease-free cultures, will exclude the parasites to a very large extent.

FOOD OF EPHESTIA KUEHNIELLA LARVAE

Although Ephestia larvae are most frequently observed in wheat flour, they are able to live in many other foods. They have been found in wheat bran, entire wheat kernels, rye flour, buckwheat

flour, corn meal, entire corn kernels, rice flour, rolled oats, cottonseed meal, prepared cereal foods, crackers, cakes, chestnuts and other nuts, cocoa beans, chocolate, dried fruits, jelly cubes, nests of the bumblebee, hives of the honeybee, and dead Ephestia and Tortrix moths. The writer has reared moths from commercial barley flour, flour made from entire hemp seed, and flour from entire field-pea seed. In two experiments with this pea flour the time taken for the first moth to emerge was 94 and 104 days, respectively, an abnormally protracted period of growth when compared with the life cycle in entire-wheat flour.

A number of attempts were made to rear moths from flour made of entire Georgia velvet beans, navy beans, lima beans, and soybeans. The moths seemed to oviposit readily in these flours, but the young larvae invariably died within a few weeks after hatching. In not one instance did a larva attain even half normal growth. It is interesting to note that McCollum, Simmonds, and Pitz (*23*) found that large quantities of navy beans (*Phaseolus vulgaris*) were injurious to rats, partly because of the low nutritive value of the proteins in the beans. Later McCollum, Simmonds, and Parsons (*24*) found that the protein of peas (*Vicia sativa*) was also inadequate for optimum growth in rats. More recently, Johns and Finks (*19*) have found that the navy-bean proteins are deficient in cystine, and that rats show only normal growth on diets containing all the protein from navy beans, provided such diets are supplemented with this amino acid. This explanation may also hold in the case of Ephestia, but that it can not be applied to insects generally is indicated by the well-known bean and pea feeding habits of the larvae of certain Coleoptera such as *Bruchus obtectus* and *B. quadrimaculatus*.

EXPERIMENTAL METHODS

Clean, sound, whole wheat was reduced to fine flour in a small hand mill. None of the coarser bran was removed, and the mositure content of the flour was not altered in any way before treatment with the solvents.

The highly milled (patent) wheat flour was a well-known commercial brand of white flour.

The Soxhlet apparatus used for most of the extractions had a capacity of 100 c. c. From 35 to 45 gm. of flour, placed in a fat-free paper extraction thimble, were treated at a time. A small number of extractions were made with a 200 c. c. Soxhlet, but the amount of flour used was the same as with the smaller apparatus. The heat was supplied by a water bath.

The methods of preparation or extraction of the food used in the individual experiments are described in the legends accompanying the figures.

The essential differences between extraction in a Soxhlet apparatus and extraction at room temperature are the higher temperature and the more frequent change of solvent in the former method. A solvent which has little or no effect on the growth value of the flour, ether for example, removes the growth-promoting substance in a much shorter time in a Soxhlet apparatus than at room temperature with several changes of solvent. On the other hand, U. S. P. chloroform when used without special purification in a Soxhlet apparatus

appears to produce destructive changes in the flour, making it unfit for growth, whereas at room temperature no such effects have been observed. It should be noted that the temperature of the solvent surrounding the flour in a Soxhlet apparatus is always below the boiling point of the solvent.

The rate of growth of Ephestia larvae was studied by comparing the length of the life cycle on an experimental food with that on a standard, i. e., entire-wheat flour. The work was done in a laboratory room. Such climatic factors as temperature, moisture, and light were not closely regulated, since apparatus for this was not available. It is believed, however, that all the experiments were affected in nearly the same degree by any fluctuations of climate that occurred during their course.

The life cycle was determined from the day on which moths or young larvae were isolated in a bottle containing the food under investigation. Only moths which had emerged within a 24-hour period were used. In the earlier experiments, a number of moths, usually four or five, were selected at random without regard to sex, but later one or several pairs of moths were employed. Unless it could be positively determined that larvae had hatched from eggs laid by the moths, the experiment was rejected. Many of the later experiments were started with a number of newly hatched larvae, usually 20, isolated on 5 gm. of flour, which furnished ample food for this number. They were obtained by isolating a pair of moths in a bottle and carefully removing the newly hatched larvae with a camel's-hair brush. Larvae hatching during 24 or 48 hours, rarely 72 hours, were selected for this purpose. In order to reduce them all to a comparable age basis, the larvae which hatched within a 48 or 72-hour interval were considered as one and two days older, respectively, than those hatching within the 24-hour interval, such time being added to the life cycle.

Daily records of the number of moths that emerged were taken, and from them the time (in days) to first emergence, last emergence, and average emergence was obtained. The last figure is the sum of the products of the life cycle in days and the number of moths emerging, divided by the total number of moths emerged.

Larvae were reared in several types of bottles. In the later experiments where the progeny of a single pair of moths or young larvae were reared, a wide-mouthed bottle 80 mm. high and 57 mm. in diameter at the base, was employed extensively. The bottles were stoppered with cotton plugs which were easily removed, but often the full-grown larvae worked their way into the cotton and pupated there. Moths have difficulty in emerging from the pupal cases in cotton and frequently die before they can free themselves. Such dead moths were always considered as normally emerged individuals.

GROWTH OF LARVAE IN NORMAL ENTIRE-WHEAT FLOUR

In order that a check on the effect of environmental factors other than food might be obtained, a control experiment with normal entire-wheat flour was run parallel to each series of experiments in which treated flour was used. The results are expressed in curves

(fig. 1) showing the length of the life cycle (obtained by the method of moving averages) for any time of the year at Washington, D. C., under the conditions governing these experiments.

Similar results were obtained for a shorter period at New York City (fig. 2). The ordinates represent the time (in days) from the isolation of moths or young larvae upon the normal flour to the emergence of moths of the new generation. The abscissae give the time of the year at which the experiments were started. Curve A (fig. 1) gives the length of the life cycle of the first moth to emerge; curve B, the average length of the life cycle of all moths; while curve C is the life cycle of the last moth to appear.

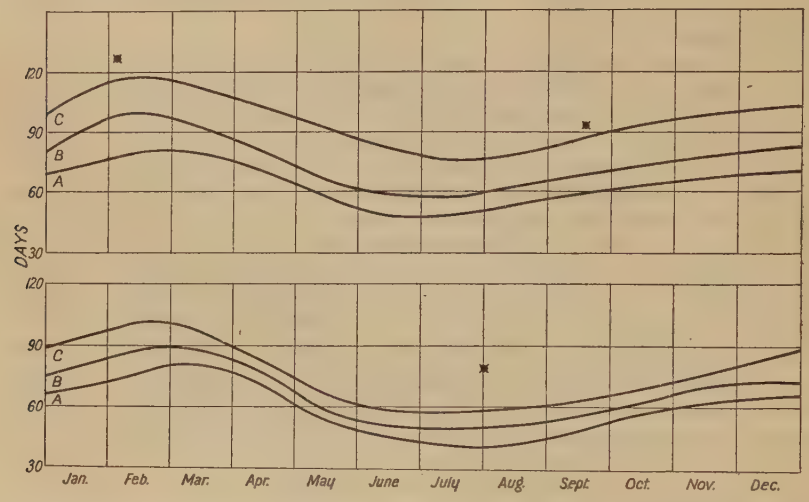

FIG. 1

Growth rate of Ephestia larvae in entire-wheat flour, as determined by the length of the life cycle. Period June, 1919, to June, 1920. Washington, D. C. Room temperature, subject to daily and seasonal fluctuations.

Upper curve.—Progeny from one or several pairs of moths placed on 10 to 15 gm. flour. (Based on 60 experiments.)

Lower curve.—Progeny from young larvae (usually 20) placed on 5 gm. flour. (Based on 99 experiments.)

A represents the length of the life cycle of the first moth which emerged, B the average time (in days) of emergence of all moths, and C the length of the life cycle of the last moth to appear.

These curves are derived from data smoothed by the method of moving averages.

The life cycle for progeny from pairs of moths grown in normal flour varied from a minimum of 46 days in summer to 85 days in winter. The average emergence varied from 54 to 105 days, and the last emergence from 64 to 124 days. Moths from newly hatched larvae required from 41 to 85 days from the first emergence, 48 to 100 days for the average emergence, and 54 to 114 days for the last emergence. These extreme deviations are not expressed in the curves in Figure 1 because of the smoothing to which the curves have been subjected.

The values for first emergence and average emergence give quite smooth curves by the method of moving averages. Both are valuable indices of the growth rate. The emergence of the last moth is more variable, and the curves (C) have been subjected to considerable smoothing.

The difference between experiments started with moths and those started with young larvae lies practically in the incubation period

of the eggs, which varies from 4 to 11 days, depending on the time of the year.

The number of moths which emerged from entire-wheat flour when the experiment was started with moths was variable, since there was often a marked difference in the number of eggs laid as well as in the proportion of those eggs which hatched. Of the experiments started with a definite number of larvae 24 or 48 hours old, involving 1,165 individuals, 76 per cent pupated and emerged as moths. A part of this mortality may have been due to injuries received while being transferred on the camel's-hair brush, but it is doubtful whether all of it can be explained in this way.

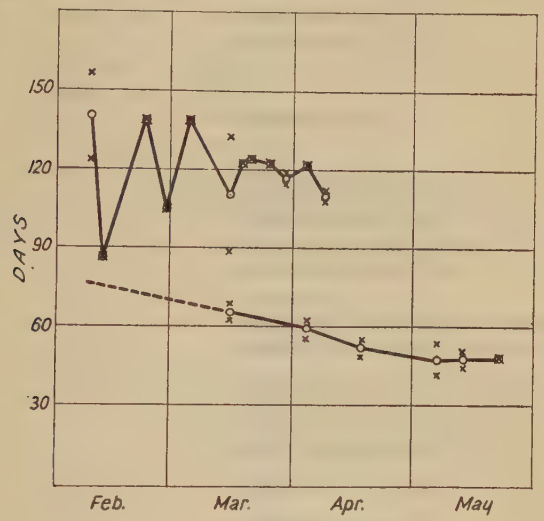

FIG. 2

Upper curve.—Days to appearance of first moth, in 18 experiments. Progeny from 4 or 5 moths placed on 10 gm. highly milled (patent) wheat flour February 8 to April 8, 1918. New York City.
Lower curve.—Days to appearance of first moth in 13 experiments. Progeny from 5 moths placed on 10 or 15 gm. normal entire-wheat flour, March 15 to May 22, 1918. New York City.
X represents a single observation; O represents the average first emergence for two or more experiments started on a given date.

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH ETHER

Entire-wheat flour was extracted with anhydrous ether at room temperature, and also in a Soxhlet apparatus, for varying lengths of time.

The effect of flour, extracted for 24 hours at room temperature, on the life cycle of Ephestia is shown in Figure 3. Experiment 2, without change of ether, is comparable to experiment 3, in which there was one change of ether during the 24 hours' extraction, and is not very different from experiments 4 and 5, in which there were two changes of ether. Although a comparison of the average time of emergence of moths in the 24-hour-extracted flour indicates a more extended period of growth than in the control, it is apparent that only a relatively small amount of the growth-promoting substance was removed.

Three or four hours' extraction in a Soxhlet apparatus does not remove much of the growth-promoting substances. Experiments 18 to 22, begun in June from pairs of moths, lost most of their larvae through disease, and all the larvae in the control were destroyed. However, they are comparable to experiments 197 to 199 (started in February from newly hatched larvae) when the controls of the respective series are considered and allowance is made for the small emergence in experiments 18 and 21. The control A was obtained from the curves in Figure 1.

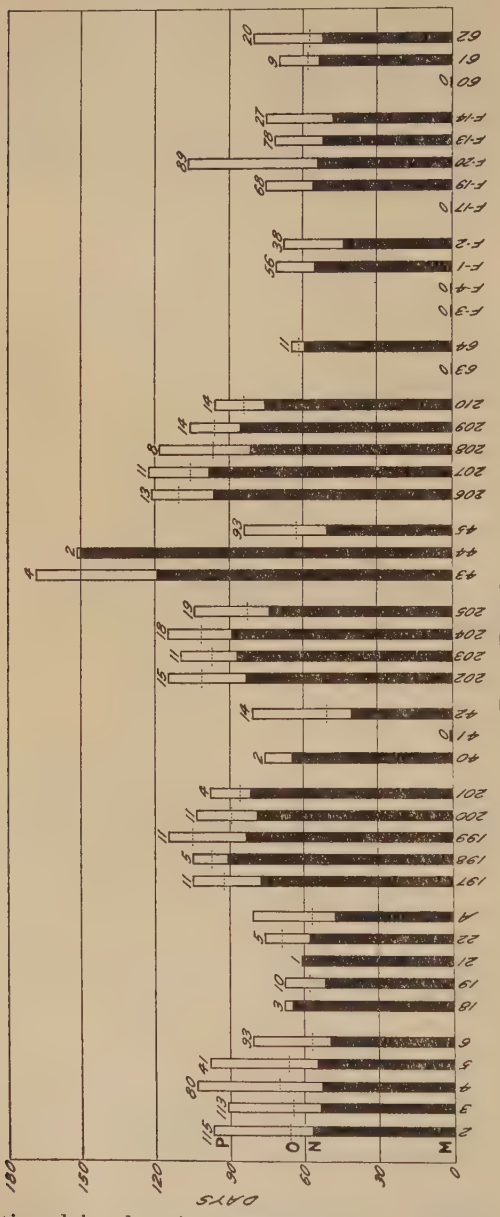

FIG. 3. (Legend on p. 905)

A 7-hour extraction in a Soxhlet apparatus effected a greater removal of the ether-soluble growth-promoting substance, but enough of the material still remained for growth. Experiments 40 and 41, commenced the last of July, gave much poorer growth than experiments 202, 203, and 204, all of which were started in February.

A 10-hour extraction in a Soxhlet apparatus likewise did not remove all of the growth-promoting substance. In experiments 206 and 207, the life cycle was markedly longer than in the control, experiment 210; when the extracted material was returned quantitatively to the extracted flour, the growth-rate (experiments 208 and 209) approached that in the control, but was exceptional in that it was still slow. Experiments 43 and 44 gave quite different results in regard to the length of the life cycle, and illustrate the variation one may expect in this kind of experimental work.

Growth generally ceased or was greatly retarded in flour that had been extracted in a Soxhlet apparatus for 15 hours or more. The best results were obtained when the Soxhlet flask was heated sufficiently to permit six ether changes per hour. In one experiment (63, fig. 3), a complete removal of the growth-promoting substance was apparently accomplished in 12 hours, but as this was not repeated it may be doubted whether growth would invariably cease in flour extracted for this length of time.

When only a few weeks old, most of the larvae died in the highly extracted flour; and the few that survived for a longer period usually failed to reach the pupal stage. If, however, the extracted material

EXPLANATORY LEGEND FOR FIGURE 3

The solid black vertical lines MN indicate the length of the life cycle (in days) from the introduction of moths or larvae in the bottle to the emergence of the first moth of the new generation. O represents the average emergence (in days) of all moths; and P the number of days to the emergence of the last moth in the experiment. The number at the top of each column represents the number of moths that have emerged in the experiment. The last line in practically every group represents either the control experiment or a duplicate of it.

2. 50 gm. entire-wheat flour extracted with 200 c. c. anhydrous ether, at room temperature, for 24 hours. 2 males, 3 females, isolated on 15 gm. flour June 4; 1 male added June 5.

3. 50 gm. entire-wheat flour extracted at room temperature, 5 hours, with 100 c. c. anhydrous ether; and 19 hours with another 100 c. c. ether. 1 male, 3 females, isolated on 15 gm. flour June 4, 1 male added June 5.

4. 50 gm. entire-wheat flour extracted at room temperature, 5 hours with 100 c. c. anhydrous ether, 16 hours with 100 c. c. ether, and 3 hours with 100 c. c. ether. 3 males, 3 females, on 15 gm. flour, June 5.

5. Duplicate of 4.

6. (Control.) 3 males, 3 females, isolated on 15 gm. normal entire-wheat flour, June 5.

18. 36 gm. entire-wheat flour extracted 3 hours, Soxhlet, with anhydrous ether. 3 males, 3 females, isolated on 15 gm. flour, June 25.

19. Duplicate of 18.

21. 36 gm. entire-wheat flour extracted 4 hours, Soxhlet, with anhydrous ether. 3 males, 3 females, isolated on 15 gm. flour, June 26.

22. Duplicate of 21. Larvae in experiments 18, 19, 21, and 22 were all badly diseased, which doubtless accounts for the small number of moths produced.

A. (Control.) Taken from curves of Figure 1. Larvae in original control died of disease.

40. 40 gm. entire-wheat flour extracted 7 hours, Soxhlet, with anhydrous ether. 20 larvae about 18 hours old isolated on 10 gm. flour, July 31.

41. Duplicate of 40.

42. (Control.) 20 larvae about 18 hours old isolated on 10 gm. normal entire-wheat flour, July 31.

43. 40 gm. entire-wheat flour extracted 10 hours, Soxhlet, with anhydrous ether. 2 males, 2 females, isolated on 15 gm. flour, August 4.

44. Duplicate of 43.

45. (Control.) 2 males, 2 females, on 15 gm. normal entire-wheat flour, August 4.

60. 33 gm. entire-wheat flour extracted 16¼ hours, Soxhlet, with anhydrous ether. 2 males, 2 females, isolated on 16 gm. flour, August 24.

61. 16 gm. flour from 60 mixed with half of the ether extract, and the ether evaporated at room temperature. 2 males, 2 females, isolated on this flour, August 24.

62. (Control.) 2 males, 2 females, isolated on 16 gm. normal entire-wheat flour, August 24.

63. 40 gm. entire-wheat flour extracted 12 hours, Soxhlet, with anhydrous ether. 1 male, 1 female, isolated on 15 gm. flour August 28; and 1 male, 1 female, isolated August 29. Larvae hatching from the eggs laid in this flour died in less than 3 months.

64. (Control.) 1 male, 1 female, isolated on 15 gm. normal entire-wheat flour, August 30.

F–1. (Control.) 5 moths isolated on 10 gm. normal entire-wheat flour, May 6.

F–2. Duplicate of F–1.

F–3. 40 gm. entire-wheat flour extracted 15 hours, Soxhlet, anhydrous ether. 5 moths isolated on 10 gm. flour, May 6.

F–4. Duplicate of F–3.

F–13. (Control.) 5 moths isolated on 15 gm. normal entire-wheat flour, May 13.

F–14. Duplicate of F–13.

F–17. 10 moths isolated on 15 gm. flour extracted 15 hours (see F–3), May 13.

F–19. 40 gm. entire-wheat flour extracted 12 hours, Soxhlet, with anhydrous ether, after which the ether extract was returned to the extracted flour quantitatively. 5 moths isolated on 15 gm. flour, May 13.

F–20. Duplicate of F–19, except that 13.5 gm. instead of 15 gm. flour was used.

197. 43.5 gm. entire-wheat flour extracted 4 hours, Soxhlet, with anhydrous ether. 20 larvae about 24 hours old isolated on 5 gm. flour, February 20.

198. Duplicate of 197.

199. Duplicate of 197.

200. (Control.) 20 larvae about 24 hours old isolated on 5 gm. entire-wheat flour, February 20.

201. Duplicate of 200.

202. 41.9 gm. entire-wheat flour extracted 7 hours, Soxhlet, with anhydrous ether. 20 larvae about 24 hours old isolated on 5 gm. flour, Feburary 21.

203. Duplicate of 202.

204. Duplicate of 202.

205. (Control.) 20 larvae about 24 hours old isolated on 5 gm. normal entire-wheat flour, February 21.

206. 44 gm. entire-wheat flour extracted 10 hours 10 minutes, Soxhlet, with anhydrous ether. 20 larvae about 24 hours old isolated on 5 gm. flour, February 24.

207. Duplicate of 206.

208. Half of the flour from 206 with half of the ether extract returned to it quantitatively, and the ether evaporated at room temperature. 20 larvae about 24 hours old isolated on 5 gm. flour, February 24.

209. Duplicate of 208.

210. (Control.) 20 larvae about 24 hours old isolated on 5 gm. normal entire-wheat flour, February 24.

containing the growth-promoting substance was returned to the residue, growth similar to that in the normal flour was obtained (F–19, F–20, 61, fig. 3), indicating that it was largely a matter of

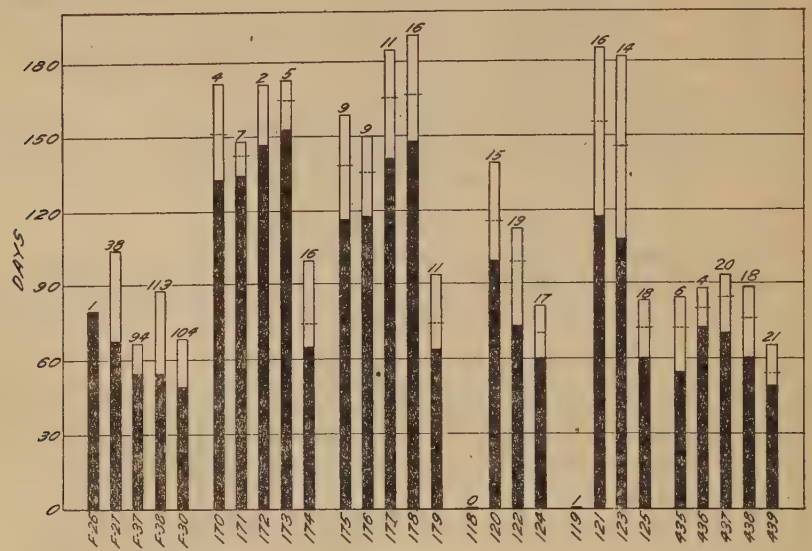

FIG. 4

F–26. 40 gm. entire-wheat flour extracted 15 hours, Soxhlet, with 95 per cent alcohol (redistilled from CaO). 10 moths placed on 15 gm. flour, May 22.

F–27. Duplicate of F–26.

F–30. (Control.) 10 moths placed on 15 gm. normal entire-wheat flour, May 22.

F–37. 40 gm. entire-wheat flour extracted 15 hours, Soxhlet, with 95 per cent alcohol (redistilled from CaO), after which the alcoholic extract was mixed with the extracted flour and the solvent evaporated at room temperature. 5 moths placed on 15 gm. flour, May 31.

F–38. Duplicate of F–37.

118. 40 gm. entire-wheat flour extracted 15 hours with anhydrous ether, and then for 10 hours with 95 per cent alcohol. Half the alcoholic extract was mixed with one-third of this extracted flour, and the solvent evaporated off at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, November 5.

119. 20 larvae 24 to 36 hours old placed November 30 and December 1 on 5 gm. of the flour used in 118. One moth emerged; life cycle 275 days.

120. One-third the flour extracted with both ether and alcohol (118) was mixed with half of the ether extract. The ether was removed at room temperature. 17 larvae about 48 hours old placed on 5 gm. flour, November 5.

121. 20 larvae about 24 hours old placed December 1 on 5 gm. of the flour used in 120.

122. One-third of the flour extracted with both ether and alcohol (118) was mixed with half of the ether, and then with half of the alcoholic extracts, the solvents being removed at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, November 6.

123. 20 larvae about 24 hours old placed December 1 on 5 gm. of the flour used in 122.

124. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, November 6.

125. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, December 1.

170. 41.8 gm. entire-wheat flour extracted 10 hours, Soxhlet, with absolute alcohol. 20 larvae about 24 hours old isolated on 5 gm. flour, January 8.

171. Duplicate of 170.

172. Half of the extracted flour from 170 mixed with half of the alcoholic extract, and the solvent evaporated off at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, January 8.

173. Duplicate of 172.

174. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, January 8.

175. 45 gm. entire-wheat flour extracted 15 hours, Soxhlet, with absolute alcohol. 20 larvae about 24 hours old placed on 5 gm. flour, January 8.

176. Duplicate of 175.

177. Half of the extracted flour from 175 mixed with half of the alcoholic extract; alcohol evaporated at room temperature. 20 larvae 24 to 48 hours old placed on 5 gm. flour, January 8.

178. Duplicate of 177, except that 25 larvae about 48 hours old were used.

179. (Control.) 18 larvae about 48 hours old placed on 5 gm. normal entire-wheat flour, January 8.

435. 50 gm. entire-wheat flour extracted with 200 c. c. 95 per cent alcohol, for 24 hours, at room temperature. 4 c. c. extract equivalent to 1 gm. extracted flour. 20 larvae about 24 hours old placed on 5 gm. flour, July 26.

436. Same as 435.

437. 15 gm. extracted flour from 435 mixed with 60 c. c. of the alcoholic extract from same, and the solvent removed at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, July 26.

438. Same as 437.

439. (Control.) 21 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, July 26.

the removal and not the destruction of a substance or substances, the absence of which was responsible for the growth failure in the extracted flour. Subsequent experiments have confirmed this view (442, 443, fig. 5).

It is believed that the failure of Ephestia larvae to grow in entire-wheat flour extracted with ether in a Soxhlet apparatus for 15 hours or more is due to the removal of vitamin A, the presence of which has long been known in the wheat kernel (*22*).

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH ACETONE

Acetone, when used at room temperature for a 24-hour period, was not an effective solvent of the growth-promoting substance in entire-wheat flour (29, 30, fig. 6). However, four hours' extraction in a Soxhlet apparatus removed enough of the substance to check the growth rate, but the food value of the residue plus the acetone extract was not equal to that of the original flour (213, 214, fig. 6). Flour subjected to continuous extraction for seven hours or more with acetone which had not been purified would not support growth at all, and the flour thus treated did not permit normal growth even when the extract was returned to it.[5]

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH BENZENE

In the series of experiments with benzene as the solvent, unless the flour was given a 15-hour period of extraction in a Soxhlet apparatus, removal of the growth-promoting substance was not sufficient to prevent growth (see fig. 7). The benzene treatment seemed to alter somewhat the food value of the flour, the life cycle always being longer on extracted flour to which the extract had been added than on normal flour. Extraction with benzene at room temperature for 24 hours apparently removed some of the growth-promoting substance, but the larvae in these experiments (26, 27, fig. 4) were so badly diseased as to leave some doubt concerning the efficiency of the solvent under these conditions.[6]

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH PETROLEUM ETHER

Entire-wheat flour was extracted with petroleum ether (b. p. 33° to 66°C.) for 9¾ and 15 hours, respectively. The results are shown in Figure 8. The larvae on extracted flour required a longer time for growth than those on the normal flour, but the removal of the growth-promoting substance was by no means complete. That all of the increase in time necessary for growth on the extracted flour was not due to the removal of a substance, however, is shown by experiments 143, 144, 151, and 152, in which growth was slower than in the con-

[5] Experiments recently completed with flour extracted with a specially purified acetone indicate that although some of the growth-promoting substance was removed, the growth value of the flour was not otherwise impaired by treatment with the solvent. The flour was extracted in a Soxhlet apparatus for 10 hours. Growth was retarded in the extracted flour, but was normal in the extracted flour plus the extract. The acetone U. S. P. was purified by allowing it to stand in contact with $CaCl_2$ for three days and distilling just before using.

[6] More recent experiments show that the extraction is not complete in 24 hours at room temperature. Flour extracted with purified benzene for 15 hours in a Soxhlet apparatus allowed slow growth, but the same flour with the extract returned had a normal growth value. The benzene C. P. was purified by shaking with concentrated sulphuric acid until the acid remained nearly colorless on standing overnight in contact with the solvent. The benzene was then shaken with sodium hydroxide solution, dried over calcium chloride, and distilled when ready for use.

trols, even when the extract was added to the extracted flour. The petroleum ether employed evidently had a deleterious action on the flour, under the conditions of these experiments.

Several experiments were made with the same lot of petroleum ether before the higher fractions were removed by distillation. Flour extracted at room temperature (50 gm. flour and 200 c. c. solvent) for 24 hours, gave 48 days for the first emergence, 58 days average emergence, and 66 days for the last emergence; or about what one would expect for normal flour. The experiment was started July 8. Ten hours' extraction in a Soxhlet apparatus allowed growth at the following rates: 57, 70, and 91 days, respectively, for the first, average, and last emergence; 55, 58, and 67 days were the corresponding rates for the control. The experiments were started August 14. The flour extracted for 15 hours in a Soxhlet apparatus, with the undistilled petroleum ether, gave rather unusual results. Growth in two duplicate experiments was as follows: 107 and 134 days, 160 and 172 days, and 199 and 205 days, respectively, for the first, average, and last emergence. The control gave 62, 69, and 75 days. This flour was extracted late in August and was not used until December 15. The unusually long period of growth probably resulted in part, at least, from the fact that the life cycle was extended into the coldest part of the year.

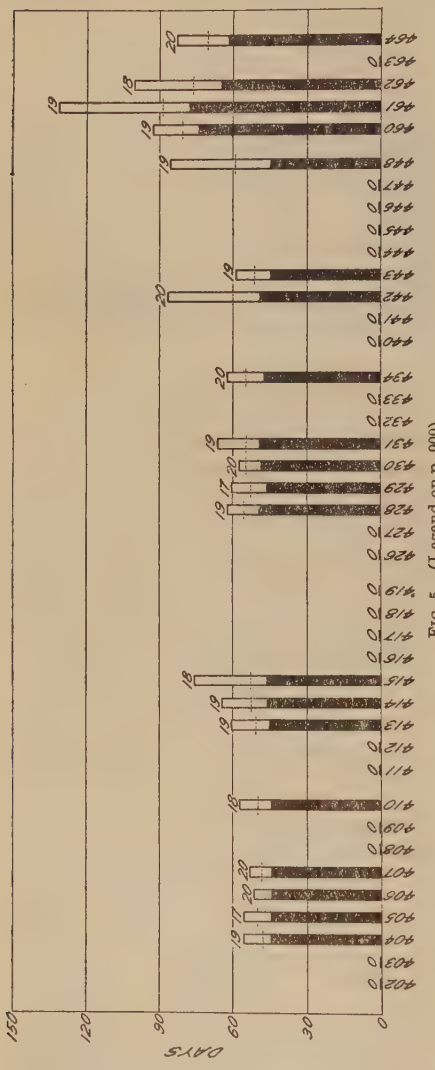

FIG. 5. (Legend on p. 909)

Since petroleum ether is a widely used fat solvent, the inference is that the growth-promoting substance incompletely extracted by it from entire-wheat flour is of the nature of vitamin A.

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH CHLOROFORM

In so far as the requirements of Ephestia larvae are concerned, removal of the growth-promoting substance was nearly complete when wheat flour was extracted with chloroform at room temperature for 24 or 25 hours. The growth rate of those moths which reached

maturity was considerably slower than that of the control moths. In experiment 37 (fig. 9), in which the larvae were badly diseased, a single moth emerged 20 days later than the single moth in the control, experiment 36. Six moths emerged from 40 larvae in experi-

EXPLANATORY LEGEND FOR FIGURE 5

402. 20 larvae about 48 hours old placed on 5 gm. entire-wheat flour extracted with chloroform U. S. P., at room temperature, for 28 hours, July 6. Flour was extracted as follows: 50 gm. flour treated with 100 c. c. solvent for 5 hours and 25 minutes, after which the solvent was removed on a Büchner funnel, and a second extraction was made with 100 c. c. solvent for 22 hours and 35 minutes. The chloroform again was removed on a Büchner funnel, and the flour was washed with two 25 c. c. portions of fresh chloroform, the washings being added to the extract. The solvent remaining in the flour was removed by evaporation at room temperature. 4.3 c. c. extract were equivalent to 1 gm. original flour.

403. Same as 402.

404. 10 gm. chloroform-extracted flour (see 402) mixed with 43 c. c. chloroform extract from the same. Solvent removed at room temperature. 20 larvae about 48 hours old placed on 5 gm. of this flour, July 6.

405. Same as 404.

406. 10 gm. chloroform-extracted flour (see 402) mixed with 10 c. c. of an ether extract of egg yolk (see 331, Fig. 13, for preparation of extract). Solvent evaporated at room temperature. 20 larvae about 48 hours old placed on 5 gm. of this flour, July 6.

407. Same as 406.

408. 10 gm. chloroform-extracted flour (see 402) mixed with 0.2 gm. olive oil dissolved in ether. 20 larvae about 48 hours old placed on 5 gm. flour, July 6.

409. Same as 408.

410. (Control.) 20 larvae about 48 hours old placed on 5 gm. normal entire-wheat flour, July 6.

411. 20 larvae about 24 hours old placed on 5 gm. entire-wheat flour extracted with chloroform U. S. P. for 24 hours, July 17. Flour extracted as follows: 50 gm. flour extracted with 100 c. c. chloroform, at room temperature, for 16 hours; solvent removed on Büchner funnel, and washed with 25 c. c. chrloroform. A second extraction was made with 100 c. c. solvent, for 8 hours, after which the solvent was removed on a Büchner funnel as before and washed with 25 c. c. chloroform. Both washings were added to the extract. The flour was freed of chloroform by evaporation at room temperature. 4.24 c. c. extract equivalent to 1 gm. original flour.

412. Same as 411.

413. 10 gm. chloroform-extracted flour (see 411) mixed with 43 c. c. chloroform extract from the same. Solvent removed at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, July 17.

414. Same as 413.

415. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, July 17.

416. 10 gm. chloroform-extracted flour (see 411) mixed with 0.5 gm. butter dissolved in ether. 20 larvae about 24 hours old placed on 5 gm. flour, July 17.

417. Same as 416.

418. 10 gm. chloroform-extracted flour (see 411) mixed with 0.5 gm. lard dissolved in ether. 20 larvae about 24 hours old placed on 5 gm. flour, July 17.

419. Same as 418.

426. 20 larvae about 48 hours old placed on 5 gm. entire-wheat flour extracted with anhydrous ether for 18 hours, Soxhlet, July 30. Flour taken from a 43-gm. lot.

427. Same as 426.

428. 10 gm. ether-extracted flour (see 426) mixed with 8 c. c. of an ether extract of egg yolk. Solvent evaporated at room temperature. The egg-yolk extract was prepared from air-dried egg yolk extracted six times with boiling ether. 1 c. c. extract contained 0.025 gm. ether-soluble substance. 20 larvae about 48 hours old placed on 5 gm. flour, July 30.

429. Same as 428, except larvae were about 24 hours old and the experiment was started July 31.

430. 10 gm. ether-extracted flour mixed with 24 c. c. of the above ether extract of egg yolk. 20 larvae about 48 hours old placed on 5 gm. flour, July 30.

431. Same as 430, except larvae were about 24 hours old. Experiment was begun July 31.

432. 10 gm. ether-extracted flour mixed with 0.2 gm. olive oil dissolved in ether. 20 larvae about 48 hours old placed on 5 gm. flour, July 30.

433. Same as 432, except larvae were about 24 hours old and experiment was started July 31.

434. (Control.) 20 larvae about 48 hours old placed on 5 gm. normal entire-wheat flour, July 30.

440. 20 larvae about 24 hours old placed on 5 gm. entire-wheat flour extracted with anhydrous ether, for 18 hours, Soxhlet, July 31. (42.3 gm. flour extracted). 1 gm. extracted flour equivalent to 3.2 c. c. ether extract.

441. Same as 440.

442. 10 gm. ether-extracted flour (see 440) mixed with 32 c. c. ether extract from same. 20 larvae about 24 hours old placed on 5 gm. flour, July 31.

443. Same as 442.

444. 10 gm. ether-extracted flour (see 440) mixed with 0.28 gm. butter dissolved in ether. 20 larvae about 24 hours old placed on 5 gm. flour, July 31.

445. Same as 444.

446. 10 gm. ether-extracted flour (see 440) mixed with 0.28 gm. lard dissolved in ether. 20 larvae about 24 hours old placed on 5 gm. flour, July 31.

447. Same as 446.

448. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour July 31.

460. 15 gm. chloroform-extracted entire-wheat flour (from a 50-gm. lot extracted 29 hours and 20 minutes with chloroform, U. S. P.; 3 changes of solvent) + 6 c. c. chloroform extract of butter oil. The butter-oil extract was prepared as follows: 50 gm. butter was dissolved in 200 c. c. absolute alcohol by heating at 50° C. for a few minutes. It was then cooled slowly to room temperature, after which the supernatant alcoholic solution was decanted from the lower less-soluble fats and insoluble material. The alcoholic solution was then evaporated nearly to dryness before a fan, at room temperature, and the residue was dried over calcium chloride for 14 days. It was then taken up in 50 c. c. chloroform U. S. P. 1 c. c. of the chloroform solution held 0.135 gm. soluble material. 20 larvae about 36 hours old placed on 5 gm. of this food, September 30.

461. Same as 460.

462. Same as 460.

463. 20 larvae about 36 hours old placed on 5 gm. of the above chloroform-extracted flour without the addition of butter oil, September 30.

464. (Control.) 20 larvae about 36 hours old placed on 5 gm. normal entire-wheat flour, September 30.

ments 358 and 359, and 59 moths from the uncounted progeny of one pair of moths in experiment 363, but in every case the growth rate was considerably retarded. Experiments 221 and 222, started with 20 larvae, each failed to produce moths. When the chloroform extract was returned to the extracted flour, growth became nearly normal. Experiments 360, 361, and 364 are in good agreement with the control, 362. Experiments 223 and 224 required a somewhat longer time for growth than the control, 225.

Flour extracted with chloroform for 24 hours at room temperature, with one change of solvent, would not permit growth of larvae to more than 3 mm. in length, and in no case did moths emerge

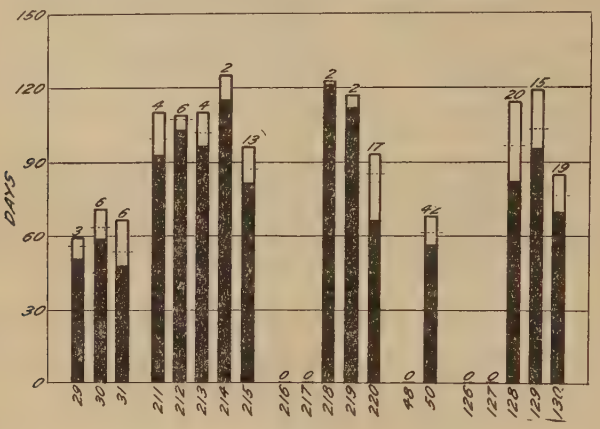

FIG. 6

48. 40 gm. entire-wheat flour extracted with acetone U. S. P., 10 hours, Soxhlet apparatus. 2 males, 2 females placed on 15 gm. flour, August 9.
50. (Control.) Entire-wheat flour. 2 males, 2 females placed on 15 gm. flour, August 9.
29. 50 gm. entire-wheat flour extracted with 200 c. c. acetone U. S. P., for 24 hours, at room temperature. 3 males, 3 females placed on 15 gm. extracted flour, July 16. (All larvae badly diseased.)
30. Duplicate of 29. (All larvae badly diseased.)
31. (Control.) Entire-wheat flour. 3 males, 3 females isolated on 15 gm. flour, July 17. (All larvae badly diseased.)
126. 39 gm. entire-wheat flour extracted with acetone U. S. P., 10 hours, Soxhlet apparatus. 20 larvae about 48 hours old placed on 5 gm. flour, December 2.
127. Duplicate of 126.
128. Half of the extracted flour from 126 mixed with half of the acetone extract, and the solvent evaporated at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, December 2.
129. Duplicate of 128.
130. (Control.) Entire-wheat flour. 20 larvae about 48 hours old placed on 5 gm. flour, December 2.
211. 45.8 gm. entire-wheat flour extracted 4 hours, acetone C. P., Soxhlet apparatus. 20 larvae about 24 hours old placed on 5 gm. flour, February 25.
212. Duplicate of 211.
213. Half of the extracted flour from 211 mixed with half of the acetone extract, and the solvent evaporated at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, February 25.
214. Duplicate of 213.
215. (Control.) Entire-wheat flour. 20 larvae about 24 hours old placed on 5-gm. flour, February 25.
216. 42.7 gm. entire-wheat flour extracted 7 hours, acetone C. P., Soxhlet apparatus. 20 larvae about 24 hours old placed on 5 gm. flour, February 26.
217. Duplicate of 216.
218. Half of the extracted flour from 216 mixed with half of the acetone extract, and the solvent evaporated at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, February 26.
219. Duplicate of 218.
220. (Control.) Entire-wheat flour. 20 larvae about 24 hours old placed on 5 gm. flour, February 23.

(402, 403, 411, and 412, fig. 5). When the chloroform extract was added to the residue, the larvae grew at a normal rate (404, 405, 413, and 414, fig. 5).

The use of chloroform at higher temperatures (in a Soxhlet apparatus) was attended by a marked lowering of the growth value of the treated flour. Experiments 226 and 227 (fig. 9), where the flour had been extracted for five hours, were practically identical with 228

and 229, where the residue had the extract returned to it. Both sets of experiments have a longer life cycle than the control 230, and a smaller average number of moths emerged from them. These experiments plainly indicate that an alteration or destruction of some substance occurred in the flour, and if the solvent removed any of the growth-promoting substance, the effects of the latter were nullified by the treatment. A 10-hour extraction in a Soxhlet apparatus prevented growth in three experiments (57, 58, and 235) and allowed very slow growth, with emergence of a few moths in one experiment (236). The residue when mixed with the extract (237 and 238) gave only a slightly shorter life cycle than the extracted

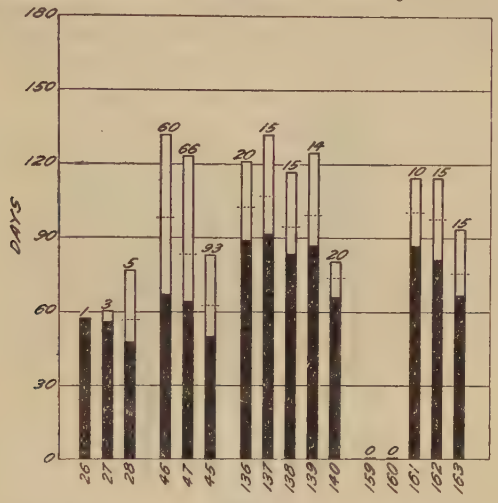

FIG. 7

45. (Control.) Entire-wheat flour. 2 males, 2 females placed on 15 gm. flour, August 4.
46. 40 gm. entire-wheat flour extracted 10 hours, benzene C. P., Soxhlet apparatus. 2 males, 2 females, placed on 15 gm. flour, August 6.
47. Duplicate of 46.
26. 50 gm. entire-wheat flour extracted with 200 c. c. benzene C. P., for 24 hours at room temperature. 3 males, 3 females on 15 gm. flour, July 9 and 10. (Larvae badly diseased.)
27. Duplicate of 26, except that experiment was started July 15. (Larvae badly diseased.)
28. (Control.) Entire-wheat flour. 3 males, 3 females placed on 15 gm. flour, July 15. (Larvae badly diseased.)
136. 41.8 gm. entire-wheat flour extracted 10 hours, benzene C. P., Soxhlet apparatus. 20 larvae about 48 hours old placed on 5 gm. flour December 13.
137. Duplicate of 136.
138. Half of the extracted flour from 136 was mixed with half of the benzene extract, and the solvent evaporated at room temperature. 20 larvae about 48 hours old placed on 5 gm. of this flour, December 13.
139. Duplicate of 138.
140. (Control.) Entire-wheat flour. 20 larvae about 48 hours old placed on 5 gm. flour, December 13.
159. 45 gm. entire-wheat flour, extraction 15 hours benzene, Soxhlet apparatus. 20 larvae about 48 hours old placed on 5 gm. flour, December 22.
160. Duplicate of 159.
161. Half of the extracted flour from 159 mixed with half of the benzene extract, and the solvent evaporated at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, December 22.
162. Duplicate of 161.
163. (Control.) Entire-wheat flour. 20 larvae about 48 hours old placed on 5 gm. flour, December 22.

flour (236), again indicating a change of some kind in the flour as a result of the extraction process. The emergence record of these experiments was also very poor.[7]

[7] Experiments made with purified chloroform since the present report was written have given different results. Flour extracted for 10 hours in a Soxhlet apparatus permitted growth at about the normal rate; and when the extracted material was added to this flour, growth was also normal. The chloroform U. S. P. was purified by shaking a number of times with 5 per cent sodium thiosulphate solution, then with distilled water. It was afterwards dried over calcium chloride and distilled just before using. Some of the effects of chloroform noted above were probably the result of impurities. Such impurities, however, did not affect the flour when the same sample of chloroform was used for the extraction at room temperature.

Chloroform was the only solvent used in these experiments which approximately removed a growth-promoting substance from entire-wheat flour in 24 hours at room temperature. The substance so extracted very probably was vitamin A.

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH CARBON TETRACHLORIDE

Entire-wheat flour was extracted with carbon tetrachloride at room temperature for 24 hours, and for 10 and 15 hours in a Soxhlet apparatus, with results which are shown graphically in Figure 10. Although many of the larvae in experiments 31 to 33 were destroyed by disease, it is certain that extraction at room temperature for a 24-hour period did not remove enough of the beneficial substance to retard growth appreciably. Ten hours' extraction in a Soxhlet

FIG. 8

141. 43.3 gm. entire-wheat flour extracted 9¾ hours with petroleum ether (b. p. 33° to 66° C.), Soxhlet apparatus. 20 larvae about 48 hours old placed on 5 gm. flour, December 13.
142. Duplicate of 141.
143. Half of the extracted flour from 142 mixed with half of the petroleum ether extract, and the solvent evaporated at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, December 13.
144. Duplicate of 143.
145. (Control.) Entire-wheat flour. 20 larvae about 48 hours old placed on 5 gm. flour, December 13.
149. 42.5 gm. entire-wheat flour extracted 15 hours with petroleum ether (b. p. 33° to 66°C.), Soxhlet apparatus. 20 larvae about 48 hours old placed on 5 gm. flour, December 15.
150. Duplicate of 149.
151. Half of the extracted flour from 149 mixed with half of the petroleum ether extract, and the solvent evaporated at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, December 15.
152. Duplicate of 151.
153. (Control.) Entire-wheat flour. 20 larvae about 48 hours old placed on 5 gm. flour, December 15.

apparatus caused a prolongation of the life cycle which was less marked in the extracted flour after the extract had been returned to it (242, 243). Flour extracted for 15 hours seemed to be still less favorable for growth, as shown by the small number of moths that reached maturity (245, 246).[8]

On the whole, carbon tetrachloride was a poor solvent for the growth-promoting substance under the conditions obtaining in these experiments.

[8] Similar results were obtained with flour which had been extracted 15 hours with purified carbon tetrachloride. The reagent was purified by shaking with concentrated sulphuric acid and allowing it to stand overnight in contact with the acid, after which it was distilled from a strong sodium hydroxide solution.

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH CARBON DISULPHIDE

Carbon disulphide did not remove an appreciable amount of the growth-promoting substance during 24 hours' extraction at room temperature (39, fig. 11). Ten hours' extraction in a Soxhlet apparatus produced a food which allowed slow growth with poor emergence

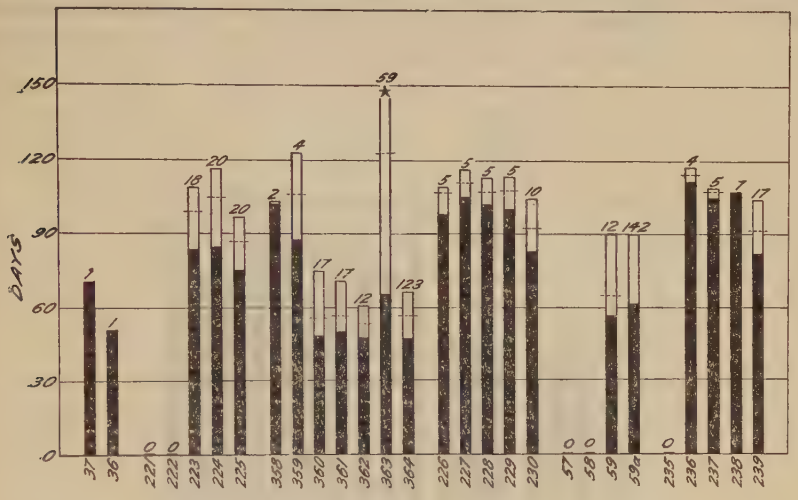

FIG. 9

57. 40 gm. entire-wheat flour extracted 10 hours, with chloroform C. P., Soxhlet. 2 fertilized females placed on 15 gm. flour, August 22. No larvae hatched from eggs laid by these females. 1 male, 1 female were placed on the same flour October 21. That larvae hatched from eggs laid by the female introduced last was evidenced by web found in the flour.

58. Duplicate of 57. Silken web was found in this flour, indicating that larvae hatched from eggs laid by the moths introduced last.

59. (Control.) 1 male, 2 females, placed on 15 gm. normal entire-wheat flour, August 22 and 23.

59a. (Control.) 1 male, 1 female, placed on 15 gm. normal entire-wheat flour, October 22.

36. (Control.) 3 males, 3 females, placed on 15 gm. normal entire-wheat flour, July 22.

37. 50 gm. entire-wheat flour extracted with 200 c.c. chloroform C. P., for 24 hours, at room temperature. 3 males, 3 females, isolated on 15 gm. flour, July 22. Larvae in 36 and 37 were very badly diseased, which accounts for the small emergence.

221. 50 gm. entire-wheat flour extracted with 200 c. c. chloroform U. S. P., for 24 hours, at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, February 26.

222. Duplicate of 221.

223. Half of the extracted flour from 221 was mixed with half of the chloroform extract, and the solvent was evaporated at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, February 26.

224. Duplicate of 223.

225. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, February 26.

226. 40.7 gm. entire-wheat flour extracted 5 hours with chloroform U. S. P., Soxhlet. 20 larvae about 48 hours old placed on 5 gm. flour, February 28.

227. Duplicate of 226.

228. Half of the extracted flour from 226 was mixed with half of the chloroform extract, and the chloroform was evaporated off at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, February 28.

229. Duplicate of 228.

230. (Control.) 20 larvae about 48 hours old isolated on 5 gm. entire-wheat flour, February 28.

235. 42.4 gm. entire-wheat flour extracted 10 hours with chloroform (purified for anesthesia), Soxhlet. 20 larvae about 48 hours old placed on 5 gm. flour, March 2.

236. Duplicate of 235.

237. Half the extracted flour from 235 mixed with half of the chloroform extract. Solvent evaporated off at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, March 2.

238. Duplicate of 237.

239. (Control.) 20 larvae about 48 hours old placed on 5 gm. entire-wheat flour, March 2.

358. 50 gm. entire-wheat flour extracted with 200 c. c. chloroform, U. S. P., for 25 hours at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, May 29.

359. Duplicate of 358.

360. Half of the extracted flour from 358 mixed with half of the extract. Solvent evaporated off at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, May 29.

361. Duplicate of 360.

362. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, May 29.

363. Larvae 24 hours old from a pair of moths placed on 14 gm. extracted flour (same as 358), May 29. *The last moth to emerge required 215 days.

364. Larvae 24 hours old from a pair of moths placed on 14 gm. extracted flour, with the extract returned (same as 360), May 29.

in two experiments (131 and 132), and no growth at all in experiments 250 and 251. After the extract was replaced in the extracted flour the life cycle was still considerably longer than that of the control in experiments 133 and 134, although emergence was much better than in the same flour without the extract. However, no growth at all occurred in 252, and only a trace of silken web was found in 253, which would indicate a little growth during the early larval stage in the latter experiment. The writer is unable to account for the lack

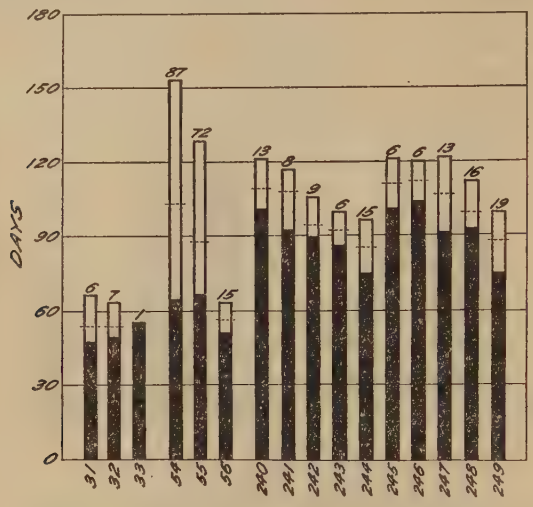

FIG. 10

31. (Control.) 3 males, 3 females, isolated on 15 gm. normal entire-wheat flour, July 17. (Larvae badly diseased.)
32. 50 gm. entire-wheat flour extracted with 200 c. c. carbon tetrachloride, C. P., for 24 hours, at room temperature. 3 males, 3 females, isolated on 15 gm. flour, July 17 and 18. (Larvae badly diseased.)
33. Duplicate of 32, July 18. (Larvae badly diseased.)
54. 40 gm. entire-wheat flour extracted for 10 hours, in a Soxhlet, with carbon tetrachloride C. P. 2 males, 2 females, isolated on 15 gm. flour, August 19.
55. Duplicate of 54.
56. (Control.) 1 male, 2 females, isolated on 15 gm. normal entire-wheat flour, August 19. The small emergence was regarded as due to disease.
240. 43 gm. entire-wheat flour extracted for 10 hours, in a Soxhlet, with carbon tetrachloride C. P. 20 larvae about 24 hours old placed on 5 gm. flour, March 3.
241. Duplicate of 240.
242. Half of the extracted flour from 240 was mixed with half of the carbon tetrachloride extract, and the solvent evaporated off at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, March 3.
243. Duplicate of 242.
244. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, March 3.
245. 42.2 gm. entire-wheat flour extracted 15 hours, Soxhlet, with carbon tetrachloride C. P. 20 larvae about 24 hours old placed on 5 gm. flour, March 3.
246. Duplicate of 245.
247. Half of the extracted flour from 245 was mixed with half of the carbon tetrachloride extract, and the solvent evaporated off at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, March 3.
248. Duplicate of 247.
249. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, March 3.

of growth in experiments 250 to 253. The carbon disulphide employed was of the same brand as that used in experiments 131 to 134, but from a different lot. No analysis of the sample used was made, but the lot from which it came was analyzed by the United States Bureau of Chemistry and found to contain no more than traces of impurities. The larvae did not grow at all in experiments 255 to 258, in which the flour was extracted for 15 hours in a Soxhlet apparatus. The carbon disulphide employed in the 15-hour extraction was from

the same container as that used for the 10-hour extraction (experiments 250 to 253).[9]

GROWTH IN ENTIRE-WHEAT FLOUR EXTRACTED WITH ETHYL ALCOHOL

Entire-wheat flour was extracted in a Soxhlet apparatus with 95 per cent alcohol for 15 hours (fig. 4). The results as shown by the length of the life cycle indicate the removal of some of the material necessary for growth, but the extraction was by no means complete

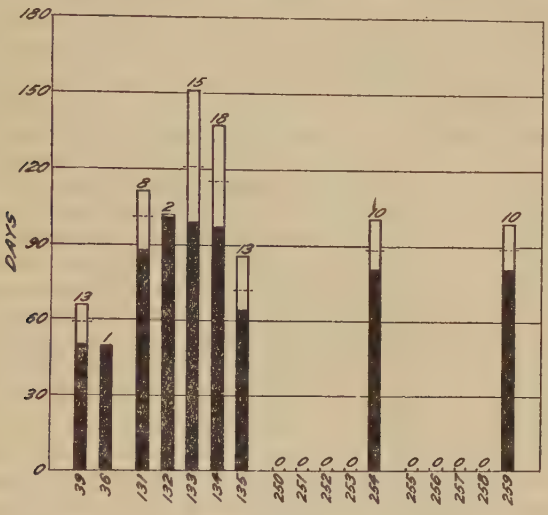

FIG. 11

36. (Control.) 3 males, 3 females, isolated on 15 gm. normal entire-wheat flour, July 22. (Diseased.)
39. 50 gm. entire-wheat flour extracted 24 hours, at room temperature, with 148 c. c. carbon disulphide C. P. 3 males, 3 females, on 15 gm. flour, July 22. (Diseased.)
131. 39.2 gm. entire-wheat flour extracted 10 hours, Soxhlet, with carbon disulphide C. P. 20 larvae about 24 hours old placed on 5 gm. flour, December 5.
132. Duplicate of 131.
133. Half of the extracted flour from 131 mixed with half of the carbon disulphide extract, and the solvent evaporated off at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, December 5.
134. Duplicate of 133.
135. (Control.) 20 larvae about 24 hours old isolated on 5 gm. normal entire-wheat flour, December 5.
250. 42.3 gm. entire-wheat flour extracted 10 hours, Soxhlet, with carbon disulphide C. P. 20 larvae about 24 hours old isolated on 5 gm. flour, March 5.
251. Duplicate of 250.
252. Half of the extracted flour from 250 mixed with half of the carbon disulphide extract, and solvent removed by evaporation at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, March 5.
253. Duplicate of 252.
254. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, March 5.
255. 43.4 gm. entire-wheat flour extracted 15 hours, Soxhlet, with carbon disulphide C. P. 20 larva about 24 hours old placed on 5 gm. flour, March 6.
256. Duplicate of 255.
257. Half of the extracted flour from 255 mixed with half of the carbon disulphide extract, solvent removed by evaporation at room temperature. 20 larvae about 24 hours old placed on 5 gm. flour, March 6.
258. Duplicate of 257.
259. (Control.) 17 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, March 6.

(experiments F–26 and F–27). The emergence also was poor in comparison to that in the control (F–30). However, the extracted flour plus the alcoholic extract showed a nearly normal growth rate (F–37

[9] Since the above was written, data have been obtained for flour extracted with purified carbon disulphide for 15 hours in a Soxhlet apparatus. Only one of 40 larvae was alive when the bottles were examined 82 days after the experiments started. The extracted flour plus extract, however, was apparently normal in its growth-promoting ability. The purified reagent was prepared from C. P. carbon disulphide, which was held in contact with anhydrous copper sulphate for two days with occasional shaking and distilled from anhydrous copper sulphate just before using. The writer is inclined to believe that the results noted in experiments 250 to 253, and 255 to 258, resulted, in part at least, from impurities, probably decomposition products, in the solvent used.

and F–38).[10] In another experiment, begun on June 19, 10 larvae about half grown (5 to 7 mm. in length) were placed on 10 gm. of the extracted flour from the same lot as that used in F–26 and F–27. The first moth to appear required 33 days for growth and transformation; or about one-half the time required by the first moth to emerge in experiment F–27, which was 68 days. Six other moths emerged later in this experiment.

The relation of the growth value of a 95 per cent alcoholic extract from entire-wheat flour (10 hours, Soxhlet) to that of an ether extract (15 hours, Soxhlet) is shown in Figure 4. In flour from which first the ether-soluble and then the 95 per cent alcohol-soluble materials were removed and the alcohol-soluble substances then replaced (118 and 119), growth continued slowly for a long time and a single moth emerged from 119 after 275 days. When the ether-soluble material only was replaced, the life cycle was prolonged, but a good emergence of moths was obtained (120 and 121). With both the alcohol-extracted and ether-extracted materials replaced, the rate of growth was still better (122 and 123). It is evident from this series of experiments that under these conditions the ether extracted more of an important growth-stimulating factor than did the alcohol.

Two series of experiments were made with entire-wheat flour extracted for 10 and 15 hours, respectively, with absolute (99+per cent) alcohol in a Soxhlet apparatus. The results are given in Figure 4 (170–179). Extraction of the growth-promoting substance was not complete in either case, and the extracted flour with the extracted material replaced was even less efficient than the extracted flour itself. It is clear that some constituent of the extracted material was changed in such a way as to check the growth of the larvae.

Cold extraction (room temperature) with 95 per cent alcohol for 24 hours resulted in an incomplete removal of the growth-promoting substance (435 and 436), and growth was still slow after the extracted material was replaced in the extracted flour (437 and 438). Thus even at room temperature, alcohol had a harmful action on the flour

GROWTH IN EXTRACTED ENTIRE-WHEAT FLOUR SUPPLEMENTED WITH FAT-SOLUBLE VITAMIN AND FATS

In order to gain an insight into the nature of the growth-promoting substance removed from entire-wheat flour by means of ether and chloroform, extracted flour was supplemented with substances rich in vitamin A, as well as substances consisting largely of fats which at most contain only small quantities of this vitamin. Butter or ether extract of egg yolk supplied vitamin A, and lard or olive oil furnished the fats, the additions forming approximately 2 per cent or more of the diet. A portion of each lot of extracted flour with the extract from it replaced was tested also to determine whether its ability to promote growth had been affected by the extraction process.

The results of these experiments are shown in Figure 5. The flour extracted at room temperature with chloroform did not permit growth to maturity (402, 403, 411, and 412), whereas the same

[10] This experiment has recently been repeated with flour extracted 15 hours in a Soxhlet apparatus with 96 per cent alcohol. The results with the extracted flour alone agree with those given above, but with the extracted flour plus extract growth was retarded as observed in the experiments with abolute alcohol. The purified alcohol was prepared from the commercial 95 per cent grade by treatment with solid sodium hydroxide for six hours, distilling and digesting the distillate with m-phenylenediamine for six days, then distilling again just before using.

flour with the chloroform extract returned to it permitted growth and transformation in practically normal time (404, 405, 413, and 414), as shown by comparison with the controls (410 and 415). The egg-yolk extract (406 and 407) rendered the extracted flour the equal of the normal untreated flour in every way. On the other hand, when butter was added to the extracted flour growth did not result, (416 and 417) and in this respect it gave the same results as olive oil (408 and 409) and lard (418 and 419).

Comparable results were obtained with the flour which was extracted with ether for 18 hours in a Soxhlet apparatus. The extracted flour would not support growth (426, 427, 440, and 441) until the extract was added to it (442 and 443), after which the growth rate was practically normal. The ether-extracted substance from egg yolk when added to the extracted flour promoted growth at a rate comparable to that in normal entire-wheat flour (428 to 431). The addition of 5.7 per cent of ether-soluble substance from egg yolk was no more effective than 2 per cent. Again butter was not effective in supporting growth (444 and 445), but gave the same negative results as olive oil (432 and 433) and lard (446 and 447).

The results obtained with butter were rather surprising, in view of the fact that it has been repeatedly used in experiments with rats as the sole source of vitamin A. That butter contains a substance detrimental to the growth of Ephestia larvae seems doubtful from the results obtained with highly milled flour supplemented with butter (fig. 13, 272, 273, 274.) Here, the life cycle was no longer than in the untreated highly milled flour. A possible explanation is that the fat-soluble vitamin in the butterfat gradually deteriorated during the course of the experiments, since freshly prepared food was not supplied after the experiments were started.[11] But the negligible amount of growth as shown by the small quantity of silken web in the flour suggests that there was not enough vitamin present at the beginning of the experiment to sustain growth for any length of time.

In order to determine whether butter contained a growth-promoting substance which would supplement the chloroform-extracted flour, a more concentrated butter preparation was sought. Butter oil, i. e., the portion soluble in cold absolute alcohol, was prepared with some modifications after the method of Osborne and Wakeman (*27*). It was added to the extracted flour at a concentration of 5.1 per cent of the resulting mixture. The results given in Figure 5 (460 to 462) indicate a satisfactory emergence of moths and a growth rate which approached but was still slower than that of the control (464). The extracted flour without butter oil did not support growth at all (463). Osborne and Mendel (*28*) observed that the growth-promoting factors were more concentrated in the butter oil than in the butter fat. These experiments agree with theirs in this respect.

The conclusion is reached from these experiments that a substance is present in the ether-extract of egg yolk which meets all the requirements of growth and the production of normal-sized moths when added to extracted entire-wheat flour that will not otherwise sustain life. It appears to be in every way as efficient as the ether and chloroform extracts of entire-wheat flour. Olive oil and lard do not

[11] Hopkins (*18*) and Drummond and Coward (*11*) have observed a considerable reduction of fat-soluble vitamin in butterfat which has been exposed to the air at ordinary temperatures.

contain this substance, or if it is present it exists in such small quantities or in such a form that it is not available for the larvae.

Butterfat also contains a substance which has a beneficial influence on growth, but it is present in a considerably lower concentration than in the egg-yolk extract. It can be obtained in a more concentracted condition in a suitable butter-oil preparation. The active substance in egg yolk and butterfat quite probably is vitamin A.

GROWTH IN HIGHLY MILLED (PATENT) WHEAT FLOUR

Early in the course of these experiments it was observed that the growth of Ephestia larvae was slower in highly milled flour than in normal entire-wheat flour. In Figure 2 the length of the life cycle of the first moth to emerge in highly milled flour (upper curve) is compared with the same life cycle in normal entire-wheat flour (lower curve). In 18 experiments with highly milled flour the first moth emerged in 110 days or more in all but four cases; the controls did not exceed 70 days in 13 experiments; and if data had been available for February they undoubtedly would have shown that not more than 85 days were required for the first emergence, a developmental time only occasionally approached by larvae grown in highly milled flour. Additional data on the growth in highly milled flour are given in Figures 12 and 13.

Not only is the baneful effect of highly milled flour shown in slow growth but the moths which reach maturity are considerably smaller than those fed normal entire-wheat flour. Sixteen males selected at random gave a mean weight of 0.0085 gm. and 13 females a mean weight of 0.0103 gm. A comparison of these figures with the weights of moths from normal flour given in Table I reveals the fact that the males from highly milled flour attain only about 60 per cent and the females about 50 per cent of the weight of the respective sexes from normal flour.

Chittenden (*9*) compared the life cycle of Ephestia larvae in highly milled flour with that in yellow-maize meal. In the former 114 days were required, in the latter 52 days. These figures are approximately the same as have been obtained in this investigation with highly milled flour and entire-wheat flour.

In regard to the food of the larvae Burkhardt (*5*) makes the following significant remarks: "Besondere Vorliebe vor allen anderen Futterstoffen zeigt die Larve für Griess, sei es von Mais, Weizen oder anderem Getreide, wie denn überhaupt die groben Vermahlungen mehr geschätzt werden als die feinen Mahlprodukte, wie beispielsweise das sog. feine Auszugmehl." The coarser wheat milling products carry most of the embryo. Its significance for growth will be discussed under the next heading.

Highly milled flour probably will not affect the growth of all grain-infesting insects in like degree. Chapman (*6*) shows that the life cycle of the confused flour beetle (*Tribolium confusum* Duval) is slightly longer in some cereal foods than in others, but apparently finds much less difference than is evident in the growth response of Ephestia.

GROWTH IN FLOUR FROM DEGERMINATED WHEAT

Highly milled wheat flour consists very largely of wheat endosperm with a small amount of bran and possibly traces of embryo present as impurities. It was thought that the relatively slow growth made

by Ephestia larvae in this flour might have been the result either of the low concentration of the bran or embryo constituents, or possibly of the paucity of both these ingredients, which are present in comparatively large amounts in the entire-wheat flour. Since only a small amount of flour was required, the embryos were removed by cutting off the tip of each kernel which contained the embryo. The wheat thus degerminated was ground in a mortar to a coarse flour that contained more than half of the branny coats of the original kernels.

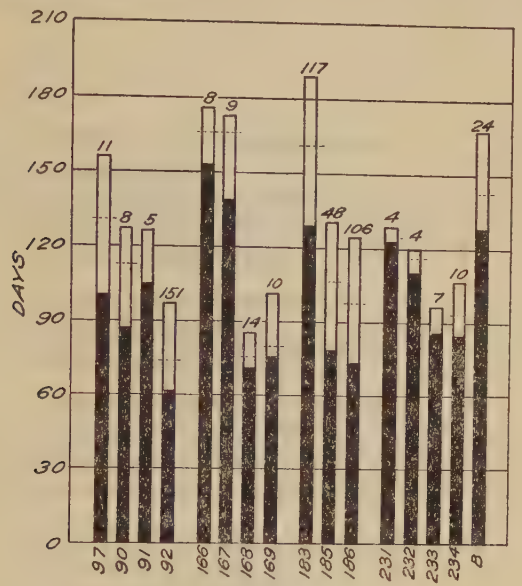

FIG. 12

90. 1 male, 1 female, placed on 15 gm. highly milled (patent) flour, October 6. (Badly diseased.)
91. Duplicate of 90. (Badly diseased.)
92. (Control.) 1 male, 1 female, placed on 15 gm. normal entire-wheat flour, October 6.
97. 1 male, 1 female, placed on 1 gm. flour from degerminated wheat, October 9. One gm. degerminated flour added on November 3 and on November 10. (Badly diseased.)
166. 20 larvae about 72 hours old placed on 5 gm. highly milled flour, December 26.
167. Duplicate of 166.
168. (Control.) 20 larvae about 72 hours old placed on 5 gm. normal entire-wheat flour, December 26.
169. Duplicate of 168.
183. 1 male, 1 female, placed on 16.3 gm. flour from degerminated wheat, February 9.
185. (Control.) 1 male, 1 female, placed on 14.1 gm. normal entire-wheat flour, February 9.
186. Duplicate of 185.
231. 20 larvae about 48 hours old placed on 2 gm. flour from degerminated wheat, March 1.
232. Duplicate of 231.
233. (Control.) 20 larvae about 48 hours old placed on 2 gm. normal entire-wheat flour, March 1.
234. Duplicate of 233.
B. Average of experiments 262, 263, 264, and 265 (fig. 13). 20 larvae about 48 hours old placed on 5 gm. highly milled flour, March 11, in each experiment.

The growth rate in the degerminated flour is shown in Figure 12, where comparison can be made with both the highly milled and the normal entire-wheat flours. Although the larvae in experiment 97 were badly diseased, the growth rate is similar to that in 90 and 91—both of which were also badly diseased—where highly milled flour was used. It is certainly much slower than the rate in normal flour (92). Experiment 183, in which no diseased larvae were observed, produced a good number of moths, but the life cycle was long, characteristic of that observed in highly milled flour when growth occurred largely during the cold part of the year (166 and 167). Experiments 231 and 232, begun on March 1, have a shorter life

cycle, since the greater part of the growth occurred during the spring. They are comparable to experiments 262 to 265, Figure 13,

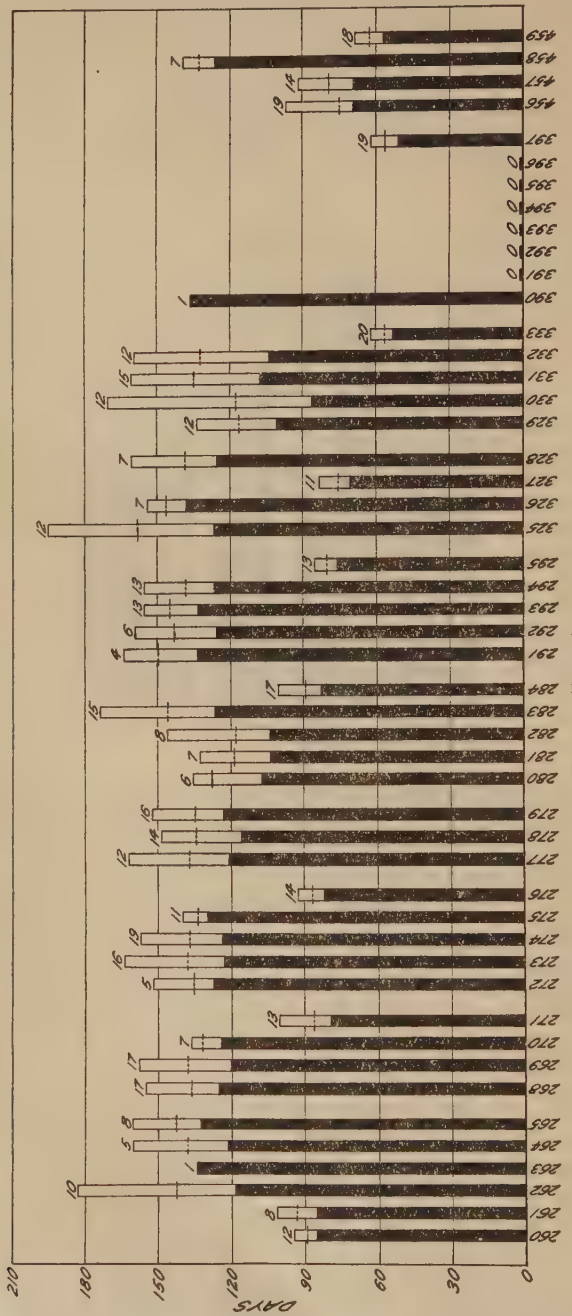

FIG. 13. (Legend on p. 921)

where the food consisted entirely of highly milled flour. These latter four experiments are averaged and shown in B in Figure 12.

EXPLANATORY LEGEND FOR FIG. 13

260. (Control.) 20 larvae about 48 hours old placed on 5 gm. entire-wheat flour, March 11.
261. Duplicate of 260.
262. 20 larvae about 48 hours old placed on 5 gm. highly milled flour, March 11.
263. Same as 262.
264. Same as 262.
265. Same as 262.
268. 16 gm. highly milled flour mixed with 30 c. c. ether extract from 45.2 gm. entire-wheat flour (15 hours, Soxhlet.); ether evaporated off at room temperature. 1 c. c. extract equivalent to 0.628 gm. entire-wheat flour. 20 larvae about 24 hours old placed on 5 gm. flour, March 16.
269. Same as 268.
270. Same as 268.
271. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, March 16.
272. 50 gm. highly milled flour mixed with 1 gm. butter dissolved in ether; solvent removed by evaporation at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, March 19.
273. Same as 272.
274. Same as 272.
275. 20 larvae about 48 hours old placed on 5 gm. highly milled flour, March 19.
276. (Control.) 20 larvae about 48 hours old placed on 5 gm. normal entire-wheat flour, March 19.
277. 50 gm. highly milled flour mixed with 1 gm. olive oil dissolved in ether; solvent removed by evaporation at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, March 19.
278. Same as 277.
279. Same as 277, except that 21 larvae were used.
280. 25 gm. highly milled flour mixed with 25 c. c. of a 50 per cent alcoholic extract of yeast. Alcohol removed by evaporation at room temperature. Yeast extract was prepared as follows: 69.3 gm. moist cakes of bakers' yeast crushed in a mortar, 30 c. c. ether being added to kill the cells. 230 c. c. of 50 per cent alcohol added gradually, after which the mixture was transferred to a flask and allowed to stand 24 hours with occasional shaking. Mixture filtered, first through cloth and then through folded paper filter. 1 c. c. of the recovered extract contained 0.0139 gm. solids. 20 larvae about 24 hours old placed on 5 gm. flour, March 20.
281. Same as 280.
282. Same as 280.
283. 20 larvae about 24 hours old placed on 5 gm. highly milled flour, March 20.
284. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, March 20.
291. 50 gm. highly milled flour mixed with 1 gm. lard dissolved in ether. Solvent evaporated at room temperature. 20 larvae about 48 hours old placed on 5 gm. flour, March 22.
292. Same as 291.
293. Same as 291.
294. 20 larvae about 48 hours old placed on 5 gm. highly milled flour, March 22.
295. (Control.) 20 larvae about 48 hours old placed on 5 gm. normal entire-wheat flour, March 22.
325. 8 gm. highly milled flour mixed with 2 gm. purified casein. Purified casein was prepared from commercial casein by dissolving in weak sodium hydroxide solution, precipitating with acetic acid, washing free of acid with distilled water by decantation, then extracting three times with hot absolute alcohol and two times with anhydrous ether. It was then dried and ground. 20 larvae about 48 hours old placed on 5 gm. mixture, April 26.
326. Same as 325.
327. (Control.) 20 larvae about 48 hours old placed on 5 gm. normal entire-wheat flour, April 26.
328. 20 larvae about 48 hours old placed on 5 gm. highly milled flour, April 26.
329. 10 gm. highly milled flour mixed with 0.2 gm. salt mixture. Composition of salt mixture: 0.1 gm. $CaCO_3$; 1.0 gm. $MgSO_4.7H_2O$; 0.5 gm KCl; 3 gm. $Na_2HPO_4.12H_2O$. 20 larvae about 24 hours old placed on 5 gm. flour plus salt mixture, May 20.
330. Same as 329.
331. 10 gm. highly milled flour mixed with 0.2 gm. salt mixture (see 329 for composition) and 20 c. c. of an ether extract of egg yolk. Preparation of egg-yolk extract: 5 gm. air-dried egg yolk finely chopped and extracted with six successive 30 c. c. portions of anhydrous ether, each portion being heated to incipient boiling. 1 c. c. of the resulting filtered ether extract contained 0.02 gm. solids. 20 larvae about 24 hours old placed on 5 gm. of this mixture, May 20.
332. Same as 331.
333. (Control.) 20 larvae about 24 hours old placed on 5 gm. normal entire-wheat flour, May 20.
390. 20 larvae about 48 hours old placed on 5 gm. highly milled flour, June 21. (Discarded by mistake after first moth emerged.)
391. 15 gm. highly milled flour mixed with 147 c. c. of a 95 per cent alcoholic extract of entire-wheat flour. Solvent removed before fan at room temperature. Extract was prepared from 30 gm. entire-wheat flour given four extractions with boiling 95 per cent alcohol, within 7 hours. 147 c. c. of extract was equivalent to 15 gm. original entire-wheat flour. 20 larvae about 48 hours old placed on 5 gm. flour, June 21.
392. Same as 391.
393. Same as 391.
394. 15 gm. highly milled flour mixed with 147 c. c. 95 per cent alcoholic extract of entire-wheat flour (see 391) and 13 c. c. of an ether extract of entire-wheat flour. Ether extract prepared from 47.7 gm. entire-wheat flour treated for 15 hours, in Soxhlet. 1 c. c. equivalent to 0.85 gm. original entire-wheat flour. 20 larvae about 48 hours old placed on 5 gm. flour, June 21.
395. Same as 394.
396. Same as 394.
397. (Control.) 20 larvae about 48 hours old placed on 5 gm. normal entire-wheat flour, June 21.
456. 25 gm. highly milled flour + 230 c. c. of a 50 per cent alcoholic extract of yeast. Evaporated to dryness before a fan at room temperature. Yeast extract prepared as follows: 71.5 gm. moist bakers' yeast was stirred into 150 c. c. boiling distilled water. Mixture was cooled, 150 c. c. absolute alcohol was added, and mixture allowed to stand 24 hours. 1 c. c. of the filtered extract contained 0.0086 gm. solids. 20 larvae 48 hours old placed on 5 gm. of this food, September 18.
457. Same as 456.
458. 20 larvae 48 hours old placed on 5 gm. highly milled flour, September 18.
459. (Control.) 20 larvae 48 hours old placed on 5 gm. normal entire-wheat flour, September 18.

Experiments 231 to 234 are interesting in another connection. Although the usual number of larvae (20) was employed, only 40 per cent of the food generally allowed was given them. The emergence was poor in both the degerminated and normal flours, but fewer than half as many moths emerged in the degerminated flour as in the latter. The facts point to the necessity for a larger amount of degerminated flour in order to complete growth and transformation, but further data will be required to substantiate them.

The weight of the newly-emerged moths from degerminated flour is apparently only a little below the average for moths from normal flour, being 0.0130 gm. for the single male weighed, and an average of 0.0200 gm. for seven females. (See Table I.)

On the basis of these experiments, it is evident that the growth rate in flour made from wheat kernels which have had the embryos removed is about the same as the rate in highly milled wheat flour. The outstanding difference seems to be the greater weight of the moths that emerge from the degerminated flour. Wheat bran carries a higher percentage of inorganic constituents than any other part of the kernel (*32, p. 427*), and one might infer that the greater gain in weight made in the degerminated flour is due to the relatively higher inorganic content of its included bran. Such a conclusion does not seem justified from the results obtained with highly milled flour supplemented with a salt mixture containing most of the inorganic constituents found in the wheat kernel. (See fig. 13.) It is possible, of course, that this salt mixture lacked some essential compound or failed to present the inorganic constituents in their proper proportions.

A substance or a group of substances present in the wheat embryo is undoubtedly responsible for the difference in the growth rate of Ephestia larvae in entire wheat and degerminated wheat flours. Osborne and Mendel (*29*) found the crude protein of wheat embryo superior to the proteins of wheat endosperm for the growth and maintenance of rats. They also have given figures which show that the embryo constitutes only 1.5 per cent of the entire kernel, and contains but 4.4 per cent of the total protein of the kernel. The results of experiments given below show quite conclusively that highly milled flour, supplemented with casein, which is known to be adequate for the growth of mammals, did not hasten the growth of Ephestia larvae. But for an almost total ignorance of the protein requirements of insects, one might conclude from the above data that the growth-promoting properties of wheat embryo were due to something besides its protein content.

Voegtlin and Myers (*33*) conclude from experiments on adult pigeons that the antineuritic vitamin is located entirely in the embyro of wheat and maize. In the case of Ephestia larvae on an exclusive diet of degerminated wheat, growth can certainly take place and apparently normal moths emerge. This difference might be explained on the basis of a quantitatively smaller amount of antineuritic vitamin required for the growth and transformation of Ephestia. If this is true, the vitamin must be derived from the peripheral layers or the endosperm of the wheat kernel, or both.

The writer has not tested the growth rate on wheat bran, but from the observations of Burkhardt (*5*) it is probable that the larvae

require a longer time for growth on this food, and transform into smaller moths which lay fewer eggs, than larvae reared in coarsely ground wheat meal (Griess).

GROWTH IN HIGHLY MILLED FLOUR SUPPLEMENTED WITH VARIOUS FOOD SUBSTANCES

From the standpoint of vertebrate nutrition, the vitamin deficiency of highly milled flour is threefold. Vitamin A is entirely lacking or reduced to a very small amount, whereas vitamin B is entirely (*33*) or largely (*8, 29, 4*) absent when the embyro is removed. Apparently the antiscorbutic vitamin is present neither in highly milled flour, nor in the entire dried grain.

Aside from its greatly reduced vitamin content, highly milled flour has lost about one-half of the fat present in entire-wheat flour, and is also deficient in mineral salts. The entire wheat kernel contains 2.18 per cent fat, and wheat endosperm contains only 1.15 per cent, bran contains 8.26 per cent, and the embryo 13.51 per cent (*29*). The ash constituents of entire wheat equal about 1.37 per cent of the edible portion of the kernel, but with the removal of the bran and embryo, which together make up only 16.5 per cent of the kernel, the remaining flour has the reduced percentage of about 0.56, or less than half the amount contained in the entire wheat (*32, pp. 423, 427*).

An attempt has been made to modify highly milled flour by the addition of various substances, which, it was thought, would correct these deficiencies. An ether extract of entire-wheat flour was added to a portion of highly milled flour to increase the vitamin A content; other lots were supplemented respectively with ether extract of egg yolk and butterfat. If the deficiency were in the nature of a fat, these substances should tend to correct it. Lard and olive oil were added to portions of highly milled flour to increase the fat content. Although they may contain some vitamin A (*10*) the amount is probably small. Vitamin B was added in the form of an alcoholic extract of bakers' yeast and an alcoholic extract of entire-wheat flour. Casein was chosen as the protein to correct any deficiency in that direction. Finally, a salt mixture was added to a portion of the flour to determine whether the deficiency in growth was due to a low concentration of any of the more abundant mineral constituents of wheat.

The results of these experiments are shown in Figure 13. The fatty substances, olive oil, lard, butter, and the ether extracts of entire-wheat flour and egg yolk, when added to highly milled flour, failed to produce a significant shortening of the life cycle of Ephestia. The olive oil, lard, and butter were used at approximately 2 per cent concentrations, the egg-yolk extract at about 4 per cent of ether-soluble substance. One gm. of the highly milled flour was used for each 1.87 c. c. of ether extract of entire-wheat flour (equivalent to 1.17 gm. original flour). There can be little doubt that the failure of Ephestia larvae to grow in this flour was due neither to a deficiency in fats nor to a deficiency in vitamin A. Some fats are already present in the milled flour, and certainly one may assume that some vitamin A is present there also. The additions to milled flour as noted above were large enough to have shown a notable increase in the growth rate if fats or vitamin A were the lacking constituents.

The weight of the moths emerging from these experiments was, like the length of the life cycle, practically identical with the weight of those from untreated highly milled flour. (See Table I.)

Neither the addition of casein nor of a salt mixture improved the growth value of highly milled flour, as both the growth rate and the weight of adult moths show (fig. 13 and Table I).

Larvae grew slowly and finally died in milled flour supplemented with the material extracted from entire-wheat flour by hot 95 per cent alcohol (391, 392, 393). Other experiments in which both an alcoholic and an ether extract of entire-wheat flour were added gave no better results (394, 395, 396). Only 3 small larvae (2.5 mm. in length) out of 120 larvae remained alive at the end of 91 days in these experimental bottles. The experiments were then discontinued.

Highly milled flour to which a 50 per cent alcoholic extract of bakers' yeast was added shortened the life cycle to a value lying between that obtained in untreated milled and normal entire-wheat flour (280, 281, and 282, fig. 13). The total solids of the yeast extract equaled 1.39 gm. in 100 c. c. The treated air-dried flour contained 1.4 per cent of the yeast solids.

The above experiment was repeated with flour in which the total solids from the alcoholic yeast extract formed 7.3 per cent of the resulting mixture. The period of growth was shorter in these experiments (456 and 457, fig. 13) than in those mentioned above, but the growth rate in relation to the control was the same. The weight of the moths was similar, the mean for eight males being 0.0131 gm. and for eight females, 0.0170 gm. (compare with Table I).

Thus it is seen that an alcoholic extract of yeast, the classical source of vitamin B, was the only substance of a number tried which improved the growth value of highly milled flour. The amount of yeast substance added to the flour, especially in the first series of experiments, was so small as to suggest strongly that the ordinary food constituents of the extract were not responsible for the improvement in growth, but that the vitamin B was.

It should be noted, however, that the life cycle in this flour was still considerably longer than in entire-wheat flour. This may have been due to an insufficient amount of vitamin B, or to a deficiency of other ingredients. It is also possible that the alcohol of the yeast extract exerted a deleterious effect upon the flour. However, in view of these results and the experience of others with higher animals, the tentative conclusion is reached that the slow growth of Ephestia larvae and the abnormal weight of the moths are due, in part at least, to the low concentration of vitamin B in highly milled wheat flour.

CONCLUSIONS

Ephestia kuehniella larvae grow normally and produce normal-sized moths in flour made from the entire wheat kernel.

In entire-wheat flour, extracted at room temperature with chloroform for a 24-hour period, only a few larvae developed into moths. Extraction under the same conditions for 24 to 28 hours, but with one or more changes of solvent, removed the growth-promoting substance so completely that the larvae died at an early age. When the material extracted with chloroform was replaced in the flour, larvae grew at nearly the normal rate.

Entire-wheat flour extracted at room temperature for a 24-hour period with ether, acetone, benzene, petroleum ether (containing fractions boiling higher than 66° C.), carbon tetrachloride, or carbon bisulphide, did not prevent or greatly prolong the period of growth of the larvae. Under the same conditions, 95 per cent alcohol extracted some of the growth-promoting substance, but in the extracted flour to which the extract was added, growth did not take place at the normal rate.

TABLE 1.—*Weight of Ephestia kuehniella moths*

Kind of flour	Males	Females
	Gm.	*Gm.*
Normal entire-wheat flour	0.0113	0.0191
	.0119	.0195
	.0124	.0196
	.0125	.0197
	.0126	.0203
	.0131	.0204
	.0132	.0205
	.0137	.0206
	.0138	.0209
	.0139	.0222
	.0140	.0224
	.0140	.0225
	.0146	.0234
	.0146	.0247
	.0146	----------
	.0149	----------
	.0151	----------
	.0154	----------
	.0159	----------
Mean weight	.0138	.0211
Highly milled flour	.0061	.0066
	.0071	.0072
	0079	.0082
	.0081	.0097
	.0084	.0099
	.0084	.0100
	.0085	.0104
	.0085	.0106
	.0086	.0107
	.0087	.0115
	.0088	.0125
	.0089	.0126
	.0091	.0138
	.0092	----------
	.0095	----------
	.0097	----------
Mean weight	.0085	.0103
Degerminated wheat flour	.0130	.0169
	----------	.0177
	----------	.0178
	----------	.0190
	----------	.0214
	----------	.0230
	----------	.0241
Mean weight	.0130	.0200
Highly milled flour, and ether extract of entire-wheat flour	.0075	.0103
	.0078	.0111
	.0085	.0112
	.0086	.0114
	.0088	.0123
	.0094	----------
	.0099	----------
	.0105	----------
Mean weight	.0089	.0113
Highly milled flour and butter	.0068	.0087
	.0071	.0093
	.0076	.0093
	.0078	.0108
	.0079	.0109
Highly milled flour and butter	0.0082	0.0110
	.0087	.0112
	.0089	----------
	.0095	----------
	.0100	----------
	.0103	----------
	.0104	----------
	.0105	----------
Mean weight	.0087	.0102
Highly milled flour, and olive oil	.0073	.0088
	.0078	.0100
	.0078	.0110
	.0079	.0110
	.0083	.0114
	.0084	.0121
	.0109	.0125
	----------	.0131
	----------	.0145
Mean weight	.0083	.0116
Highly milled flour, and alcoholic extract of yeast	.0129	.0159
	.0129	.0176
	.0134	.0191
	.0137	.0216
	.0143	----------
Mean weight	.0134	.0185
Highly milled flour, and lard	.0070	.0098
	.0076	.0101
	.0082	.0114
	.0095	----------
Mean weight	.0081	.0104
Highly milled flour, and casein	.0056	.0089
	.0062	.0093
	.0079	.0106
	.0088	.0128
	.0091	----------
	.0098	----------
Mean weight	.0079	.0104
Highly milled flour, and salt mixture	.0073	.0114
	.0076	.0115
	.0088	.0116
	.0092	.0132
	.0096	.0141
	.0129	.0147
Mean weight	.0092	.0127
Highly milled flour, mixed with ether extract of egg yolk and salt mixture	.0067	----------
	.0081	----------
	.0084	.0087
	.0087	.0117
	.0087	.0144
	.0091	.0147
	.0093	----------
	.0099	----------
	.0103	----------
Mean weight	.0088	.0124

Entire-wheat flour extracted with anhydrous ether for 15 hours or more in a Soxhlet apparatus sometimes permitted slow growth, but the larvae nearly always failed to reach the pupal stage. The extracted flour with the ether-extracted material returned to it was almost equal to the untreated flour in ability to promote growth.

Entire-wheat flour extracted in a Soxhlet apparatus with a number of other solvents gave the following results: A 10-hour treatment with purified acetone was insufficient to remove all the growth-promoting substance. Extraction with purified benzene for 15 hours also was insufficient. Neither petroleum ether (b. p. 33° to 66° C.) nor carbon tetrachloride completely extracted the growth-promoting substance in 15 hours. Extraction for 10 hours with purified chloroform permitted growth at about the normal rate. Purified carbon disulphide removed enough growth-promoting material in a 15-hour extraction to prevent moths from emerging. Ninety-five per cent and absolute ethyl alcohol affected the flour in such a way that growth was retarded after 15 hours' extraction, but moths could still emerge from it. When the 95 per cent alcoholic extract was replaced in the extracted flour, the food was nearly normal in two experiments. However, replacement of the material extracted with absolute alcohol resulted in slower growth than in the extracted flour.

The experiments indicate that certain solvents of standard grade, when used in a Soxhlet apparatus, may contain impurities which will affect the growth value of the extracted flour.

An ether extract of egg yolk when added to entire-wheat flour extracted for 18 hours in a Soxhlet apparatus with anhydrous ether permitted the growth of larvae at the normal rate.

Entire-wheat flour extracted with chloroform at room temperature for 28 hours with one change of solvent, and supplemented with an ether extract of egg yolk, promoted normal growth of Ephestia larvae.

Entire-wheat flour extracted with anhydrous ether for 18 hours in a Soxhlet apparatus, or with chloroform at room temperature for 24 hours, with several changes of solvent, and then supplemented with the ether-soluble part of butter (2.7 to 4.8 per cent), did not permit growth.

Entire-wheat flour when thoroughly extracted with chloroform at room temperature and supplemented with butter oil (i. e., the part of butterfat which is soluble in cold absolute alcohol) permitted growth at a fairly rapid but still subnormal rate.

Entire-wheat flour extracted with ether or chloroform, and supplemented with lard or olive oil, was inadequate for growth.

Highly milled (patent) wheat flour caused a retardation in the growth rate and the production of moths weighing from approximately 50 to 60 per cent of the normally fed moths.

Flour made from wheat kernels with the embryos removed, but containing more than half of the branny substance of the original seed, promoted growth at approximately the same rate as the highly milled flour. The moths which emerged from it, however, were of nearly the same weight as those maturing in normal entire-wheat flour.

Highly milled flour supplemented with olive oil, lard, butter, and ether extracts of egg yolk and of entire-wheat flour, showed no improvement in the rate of growth or the weight of the moths over

the untreated highly milled flour. When supplemented with a hot 95 per cent alcoholic extract of entire-wheat flour, moths did not emerge from the flour. The addition of an alcoholic and an ether extract of entire-wheat flour led to the same result.

Highly milled flour supplemented with purified casein, or a salt mixture containing most of the mineral constituents of wheat, did not permit more rapid growth than the highly milled flour itself.

Highly milled flour containing 1.4 per cent solids from a 50 per cent alcoholic extract of bakers' yeast, increased the growth rate and the weight of the moths to values lying between those for entire-wheat flour and highly milled flour. When the flour contained 7.3 per cent of yeast solids, the growth rate and weight of the moths were about the same as observed in the flour with the smaller content of yeast solids.

It is believed that ether and chloroform both remove a substance or substances from entire-wheat flour which are similar to, or identical with, vitamin A as understood in mammalian nutrition.

Egg yolk and butter contain a substance which promotes the growth of Ephestia larvae. It is quite probably vitamin A. It seems to be more abundant or more stable in egg yolk than in butterfat.

Since the growth value of highly milled wheat flour was considerably improved by the addition of an alcoholic extract of yeast, it is believed that the deficiency of flour for growth lies, at least partly, in its low concentration of vitamin B.

The quantity of vitamin B necessary for the growth of Ephestia at a normal rate appears to be contained largely, but not entirely, in the embryo of the wheat kernel.

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